THE UNIVERSITY OF ALBERTA

Pharmacokinetics and Pharmacodynamics of Tetrabenazine in Tardive Dyskinesia

by

Reza Mehvar

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN

PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy

IN

Pharmaceutical Sciences (Pharmacokinetics)

Faculty of Pharmacy and Pharmaceutical Sciences

EDMONTON, ALBERTA

Spring 1988

Permission has been granted to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film.

The author (copyright owner) has reserved other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without his/her written permission.

L'autorisation a été accordée à la Bibliothèque nationale du Canada de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur (titulaire du droit d'auteur) se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation écrite.

ISBN 0-315-42920-8

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR Reza Mehvar

TITLE OF THESIS Pharmacokinetics and Pharmacodynamics of Tetrabenazine in Tardive Dyskinesia

DEGREE Doctor of Philosophy

YEAR THIS DEGREE GRANTED Spring 1988

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(SIGNED) R. Mehvar

PERMANENT ADDRESS:

435 Yousef-Abad Ave., Tehran, Iran

.....

.....

.....

Date: April 25, 1988

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and research for acceptance, a thesis entitled "Pharmacokinetics and Pharmacodynamics of Tetrabenazine in Tardive Dyskinesia" submitted by Reza Mehvar in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Pharmaceutical Sciences (Pharmacokinetics).

Fakhr. Jamali
.....
Supervisor

R. T. Coutts
.....

.....

.....

.....
External Examiner

Date: April 25, 1988

To my wife,

Nasrin

ABSTRACT

Tardive dyskinesia (TD) is considered as a late-onset side effect of neuroleptic therapy which is characterized by hyperkinetic movements, occurring most often in the oral region. Treatment of TD has proven to be difficult, as most of the drugs which have been tried failed to be both effective and safe over an extended period of time.

Tetrabenazine (TBZ) appears to be the drug of choice in the control of TD and Huntington's chorea. The treatment with TBZ, however, has been empirical, as virtually no information has been available regarding its pharmacokinetics.

To study pharmacokinetics and pharmacodynamics of the drug and its major metabolite, dihydrotetrabenazine (DHT), an HPLC assay was developed. The method involves protein precipitation of the biological specimen and oxidation of the compounds with mercuric acetate at 110°C for 1 h. Fluorescence detection was utilized at excitation and emission wavelengths of 265 and 418 nm, respectively. Components of the samples were best resolved at 60°C using a mobile phase of water:acetonitrile:acetic acid:triethylamine (65:33:2:0.15) at a flow rate of 0.6 mL/min. Minimum quantifiable concentrations of the drug and its dihydro metabolite were 0.5 and 2.0 ng/mL, respectively.

Pharmacokinetics of the drug and its dihydro metabolite were studied in four patients affected by TD and in the rat. In the patients, the steady-state area under the plasma concentration-time curves (AUCs) of the metabolite were 82.6-199-fold higher than those

of TBZ. The drug showed a low oral availability of 6% in humans and 17% in rats, due to an extensive first-pass metabolism in the liver. In both species, the drug underwent a very extensive metabolism. It seems that the metabolite also undergoes sequential metabolism in the liver. Pharmacokinetics of TBZ and DHT were shown to be linear over a dosage range of 0.5-10 mg/kg of TBZ, administered orally to rats.

Pharmacodynamic studies of TBZ and DHT were conducted in the rat. Consistent with the available data in the patients, a direct relationship was observed between serum or brain concentrations of DHT and pharmacologic effects. This indicates that the observed activity following TBZ injection was mainly, if not totally, due to formation of the active metabolite, DHT. Furthermore, time courses of TBZ and DHT in the brain (site of action) were parallel to those in serum.

ACKNOWLEDGMENTS

The author is greatly indebted to Dr. F. Jamali for the opportunity to study under his guidance. Dr. Jamali's valuable time and advice were always generously provided.

The author wishes to acknowledge the following individuals: Drs. F. M. Pasutto and Y. K. Tam of the Faculty of Pharmacy, University of Alberta for interpretation of mass spectra and the use of liver perfusion facilities, respectively; Dr. G. B. Baker of the Department of Psychiatry, University of Alberta, for the use of his laboratory for the assay of brain amines; and Ms. C. H. Lemko for her technical assistance. Very helpful and valuable comments of members of the examining committee are greatly appreciated.

Provision of Alberta Heritage Foundation for Medical Research Studentship to the author is also acknowledged.

TABLE OF CONTENTS

CHAPTER	Page
1. INTRODUCTION	1
1.1. The Drug	1
1.1.1. History	1
1.1.2. Physiochemical Properties	1
1.1.3. Mechanism of Action	3
1.1.4. Side Effects and Toxicity	4
1.1.5. Pharmacokinetics and Metabolism	5
1.2. The Disease	8
1.2.1. History	8
1.2.2. Clinical Features	8
1.2.3. Prevalence and Risk Factors	9
1.2.4. Pathophysiologic Basis of Tardive Dyskinesia	10
1.2.4.1. Dopamine Receptors	10
1.2.4.2. Dopamine Mechanisms	11
1.2.4.3. Nondopaminergic Mechanisms	12
1.2.4.3.1. GABA Dysfunction	12
1.2.4.3.2. Other Neurotransmitters	13
1.2.5. Treatment of Tardive Dyskinesia	14
1.2.5.1. Dopaminergic Approaches	14
1.2.5.2. Nondopaminergic Approaches	15
1.3. Hypothesis	18
1.4. Objectives	18
1.5. References	19

2. Direct Injection High Performance Liquid Chromatotography of Tetrabenazine and Its Metabolites in Plasma of Humans and Rat..25
2.1. Introduction.....25
2.2. Experimental Section.....26
2.2.1. Materials.....26
2.2.2. Apparatus.....26
2.2.3. Standard Solution.....26
2.2.4. Sample Preparation.....27
2.2.5. Chromatography.....28
2.2.6. Patient Study.....29
2.2.7. Animal Study.....30
2.2.8. Validation Test.....30
2.3. Results and Discussion.....31
2.4. References.....46
3. Pharmacokinetics of Tetrabenazine and Its Major Metabolite in Man and Rat. Bioavailability and Dose-Dependency Studies.....48
3.1. Introduction.....48
3.2. Experimental Section.....49
3.2.1. Materials.....49
3.2.2. Patient Study.....49
3.2.3. Animal Experiments.....49
3.2.4. Blood:Plasma Concentration Ratio.....51
3.2.5. Assay Procedure.....52
3.2.6. Pharmacokinetic Analysis.....53
3.3. Results.....54
3.3.1. Patient Study.....54
3.3.2. Animal Experiments.....54

3.3.3. Blood:Serum TBZ Concentration Ratio.....60
3.4. Discussion.....64
3.5. References.....69
4. Concentration-Effect Relationships of Tetrabenazine and Dihydrotetrabenazine in the Rat.....72
4.1. Introduction.....72
4.2. Experimental Section.....73
4.2.1. Materials.....73
4.2.2. Doses and Sample Collection.....73
4.2.3. Assay Procedure.....74
4.2.4. Data Analysis.....75
4.3. Results and Discussion.....77
4.4. References.....85
5. General Discussion and Conclusions.....94

LIST OF TABLES

Table		Page
2-1.	Compounds tested for their appropriateness as an internal standard	38
2-2.	Accuracy and precision of the fluorescence-HPLC assay	40
2-3.	Comparison of UV- and fluorescence-HPLC methods	41
3-1.	Characteristics of patients and TBZ dosage regimen	50
3-2.	Pharmacokinetic indices of TBZ and DHT in patients under treatment with TBZ	56
3-3.	Pharmacokinetic indices of TBZ in the rat	61
3-4.	Pharmacokinetic indices of DHT in the rat	63
4-1.	Regression parameters obtained from the plots of %effect versus time	84
4-2.	Maximum effect and equilibrium dissociation constant values after administration of TBZ or DHT	87

LIST OF FIGURES

Figure		Page
1-1.	Chemical Structures of TBZ and DHT	2
1-2.	Suggested metabolic pathways of TBZ	7
2-1.	Chromatograms of fluorescent-HPLC assay in man	34
2-2.	Chromatograms of UV- and fluorescent-HPLC assays in the rat	36
2-3.	Effect of addition of TEA to the mobile phase on the shape of the standard curve	37
2-4.	Plasma profiles of TBZ and DHT in the rat	43
2-5.	Plasma profiles of TBZ and DHT in two patients	44
3-1.	Plasma profiles of TBZ and DHT in one patient	55
3-2.	Chromatograms of inlet and outlet samples in the liver perfusion study	57
3-3.	Plasma profiles of TBZ and DHT in rats after oral and iv administration of TBZ	58

3-4. Plots of AUC versus dose.....59

3-5. Plasma profiles of DHT in the rat after administration of the preformed metabolite.....62

4-1. Chromatograms of brain samples.....77

4-2. Brain and plasma profiles of TBZ and DHT.....78

4-3. Brain profiles of monoamines.....81

4-4 Response-log concentration curves.....83

4-5 Concentration/effect-concentration plots.....86

GLOSSARY OF ABBREVIATIONS

ANOVA	Analysis of Variance
AUC	Area Under plasma concentration-time Curve
AUC_{iv}	AUC after iv administration
AUC(m)	AUC of a preformed metabolite after iv administration
AUC(m,D)	AUC of a metabolite formed after iv administration of its precursor
AUC(m)o	AUC of a preformed metabolite after oral administration
AUC_o	AUC After oral administration
BBB	Blood-Brain Barrier
C	Concentration
Cl_o	Oral clearance
Cl_s	Systemic Clearance
C_{max}	Maximum plasma concentration
°	degrees Celcius
CI	Chemical Ionization
DA	Dopamine
DHT	Dihydrotetrabenazine
e	extraction ratio
E	Effect
E_{max}	maximum effect
f	Oral bioavailability
F_m	Fraction of a dose converted to a metabolite

GABA	Gamma-Aminobutyric Acid
5-HT	serotonin
h	hour(s)
HPLC	High-Performance Liquid Chromatography
IS	Internal Standard
ip	intraperitoneal
iv	intravenous
K_d	equilibrium dissociation constant
kg	kilogram(s)
K_i	inhibitory constant
L	litre(s)
mg	milligrams
mg/L	milligrams per litre
min	minute(s)
mL	millitre
n	number of observations
NE	Norepinephrine
ng	nanogram
po	oral
Q	Liver blood flow
r	correlation coefficient
SD	Standard Deviation
sec	second(s)
$t_{1/2}$	half-life
TBZ	Tetrabenazine
TD	Tardive Dyskinesia
TEA	Triethylamine

tid	three times a day
T_{max}	Time to peak maximum plasma concentration
UV	Ultraviolet
V_d	Volume of distribution
Yr	Year

1. INTRODUCTION

1.1. The Drug

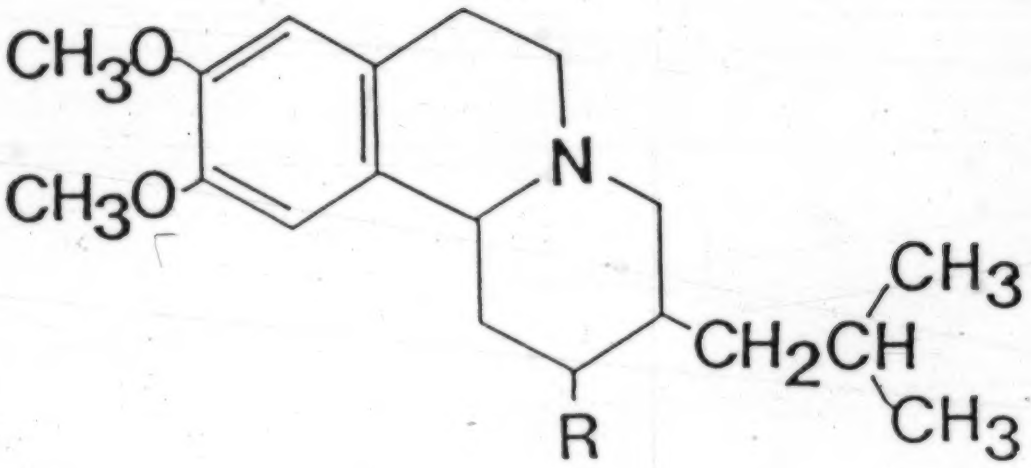
1.1.1. History

While studying the emetine derivatives, in 1956, Brossi et al. synthesized a new group of drugs, benzoquinolizine derivatives (1). It was further shown that some of the synthesized derivatives were capable of depleting brain levels of norepinephrine (NE), dopamine (DA), and serotonin (5-HT) in various animal species (2-10). In 1959 tetrabenazine (TBZ), the prototype of this class of drugs, was marketed as an antipsychotic agent in Switzerland (11). By 1963, the drug was also licensed in other countries such as England, The Netherlands, and Finland (12). Tetrabenazine was considered to be similar to reserpine (RES), but with fewer side effects (4,11).

As more effective antipsychotics were introduced in the late 50's and 60's, TBZ was withdrawn from the market in most of the countries. However, at the same time, reports began to appear indicating its usefulness in the treatment of various movement disorders, including chorea (13), drug-induced dyskinesia (14), spinal myoclonus (15), dystonia (16), hemiballismus (17), and tardive dyskinesia (TD) (18). It is now considered to be the drug of choice in the control of Huntington's chorea (13) and TD (19).

1.1.2. Physiochemical Properties

The chemical structures of tetrabenazine, 1,3,4,6,7,11b-hexahydro-3-isobutyl-9,10-dimethoxy-2H-benzo(a)quinolizin-2-one, and its dihydrometabolite are shown in Figure 1-1.



1 R, =O

2 R,

Fig. 1-1. Chemical structures of TBZ (1) and its dihydrometabolite (2).

Tetrabenazine is a white, crystalline substance with a bitter taste. The pK_a of TBZ and DHT are 6.0 and 7.5, respectively. The drug is marketed as its methanesulfonate salt with a melting point of 126-130°C. This salt is sparingly soluble in water, but dissolves freely in methanol.

1.1.3. Mechanism of Action

Pletcher et al. (4) reported a rapid and significant decrease in the brain levels of NE and 5-HT following administration of TBZ to mice, rats, guinea pigs, and rabbits, an effect similar to that of reserpine. The two drugs, however, are different in several aspects (4): 1) the duration of action of TBZ (<24 h) is much shorter than that of RES (several days), 2) reserpine is 10-20 times more potent than TBZ, and 3) recently it has been shown (20) that TBZ, but not RES, in addition to depletion of brain monoamines, is capable of blocking both pre- and postsynaptic dopamine (DA) receptors.

After release into the synaptic cleft, DA and NE are taken up into the presynaptic neurone by a sodium-dependent ATPase mechanism (21). The amines are then stored in the storage vesicles inside the presynaptic neurone, through a magnesium-dependent ATPase. Tetrabenazine and RES disrupt the stability of the amine-ATP-protein-magnesium ion storage complex by chelating magnesium ion (21). Consequently, uptake of the amines into the storage vesicles is reduced. Furthermore, leakage of the amines into the cytoplasm is followed by their destruction by MAO. The sodium-ATPase-dependent process of neuronal uptake of DA and NE, however, is not affected by either of the drugs. The same mechanism

appears to be responsible for depletion of the brain 5-HT (21). While the effects of TBZ on the storage vesicles is reversible, RES appears to act irreversibly. Hence, the recovery of the amine levels in the brain after RES administration is slow (4).

1.1.4. Side Effects and Toxicity

The results of different animal (4,12) and human (11,13,14,16,22,23) studies indicate that TBZ is a relatively safe drug even after chronic administration. The effects of TBZ on the peripheral NE and 5-HT, unlike those of RES, appear to be insignificant (4). Consequently the common side effects of RES, such as hypotension, normally have not been observed after TBZ administration. In most of the short term studies with TBZ, side effects have been absent (14) or minimal (11,13,16,22) consisting of fatigue, confusion, sweating, amenorrhea, drowsiness, insomnia and parkinsonism. The side effects disappear following reduction of the dose in most of the cases, and upon drug withdrawal in almost all the cases. Even relatively high doses of up to 225 mg/day of TBZ have not been associated with any serious side effects (23).

A relatively recent long-term study (22) on 124 patients under treatment with TBZ for various hyperkinetic disorders revealed that the side effects were infrequent and usually mild. The dose of TBZ in these patients ranged from 12.5 to 225 mg/day, and the majority of the patients were observed for at least 12 months. Some of the patients was under treatment with TBZ for 15 years. Twenty one percent of the patients experienced one or more side effects, fatigue being the most frequent one. In five cases, the side effects

necessitated discontinuation of the therapy. It was concluded that long-term TBZ therapy seemed to be safe.

After ingestion of 40 tablets (1 g) of TBZ in one case of self-poisoning (24), only drowsiness and sweating were noted. The state of consciousness of the patient improved after 24 h and she talked rationally and gained full control of micturition after 72 h.

The LD_{50} of the drug in mice has been reported to be 150 mg/kg by iv injection, 400 mg/kg by sc injection, and about 550 mg/kg by po administration (25).

1.1.5. Pharmacokinetics and Metabolism

Despite appearance of relatively numerous clinical reports in the literature investigating the effectiveness of the drug in a variety of movement disorders, the reports on the pharmacokinetics and metabolism of the drug have been scarce. The first article of such kind was reported by Quinn et al. in 1959 (2). As high as 50 mg/kg intravenous doses of TBZ were administered to rabbits, and the drug and its metabolites were measured in biological fluids fluorometrically. These authors showed that in rabbits, the drug undergoes an extensive metabolism, as only less than 2% of unchanged drug was excreted in the urine. The drug entered the brain rapidly and reached its maximum concentration within 10 min. Twenty-four h after drug administration, it could not be detected in the body. Soon after TBZ administration, the drug appeared in various tissues, including heart, small intestine, liver, kidney, lung, muscle, spleen, and fat; tissue concentrations were higher than those in plasma in most of the cases.

In 1966 Schwartz et al. (26) reported the results of their studies on the metabolic pathways of TBZ in the rabbit, dog and man. Following administrations of TBZ to the different species, urine was collected, and TBZ and its conjugated and unconjugated metabolites were extracted from the urine. Following thin layer chromatographic analysis, the metabolites were visualized utilizing mercuric acetate at 110^0 C. The authors then proposed the following routes as major metabolic pathways of TBZ in all three species studied (Fig. 1-2):

1. Reduction of the keto group at C_2, forming the dihydro metabolite.
2. Selective ether cleavage at C_9, followed by conjugation of the resultant phenolic group.
3. Oxidation at position 2′ of the isobutyl side chain.

The dihydrometabolite (DHT) may also undergo further metabolism at the above mentioned sites.

In a relatively recent article (27), using gas chromatographic-mass spectrometric technique, two other metabolites of TBZ, bis-desmethyl- and bis-desmethyl-hydroxy TBZ, were reported in the urine of psychiatric patients. This suggests that ether cleavage also takes place at C_{10}. This metabolite can then undergo oxidation at position 2′ of the isobutyl side chain, resulting in formation of bis-desmethyl-hydroxy TBZ.

Very recently, Roberts et al. (28) reported pharmacokinetics of TBZ and DHT in patients affected by TD or Huntington's chorea. This article is discussed in detail in chapter 3.

In terms of binding to plasma proteins, values of 83-88% for the drug and 44-59% for the dihydro metabolite have been reported (28).

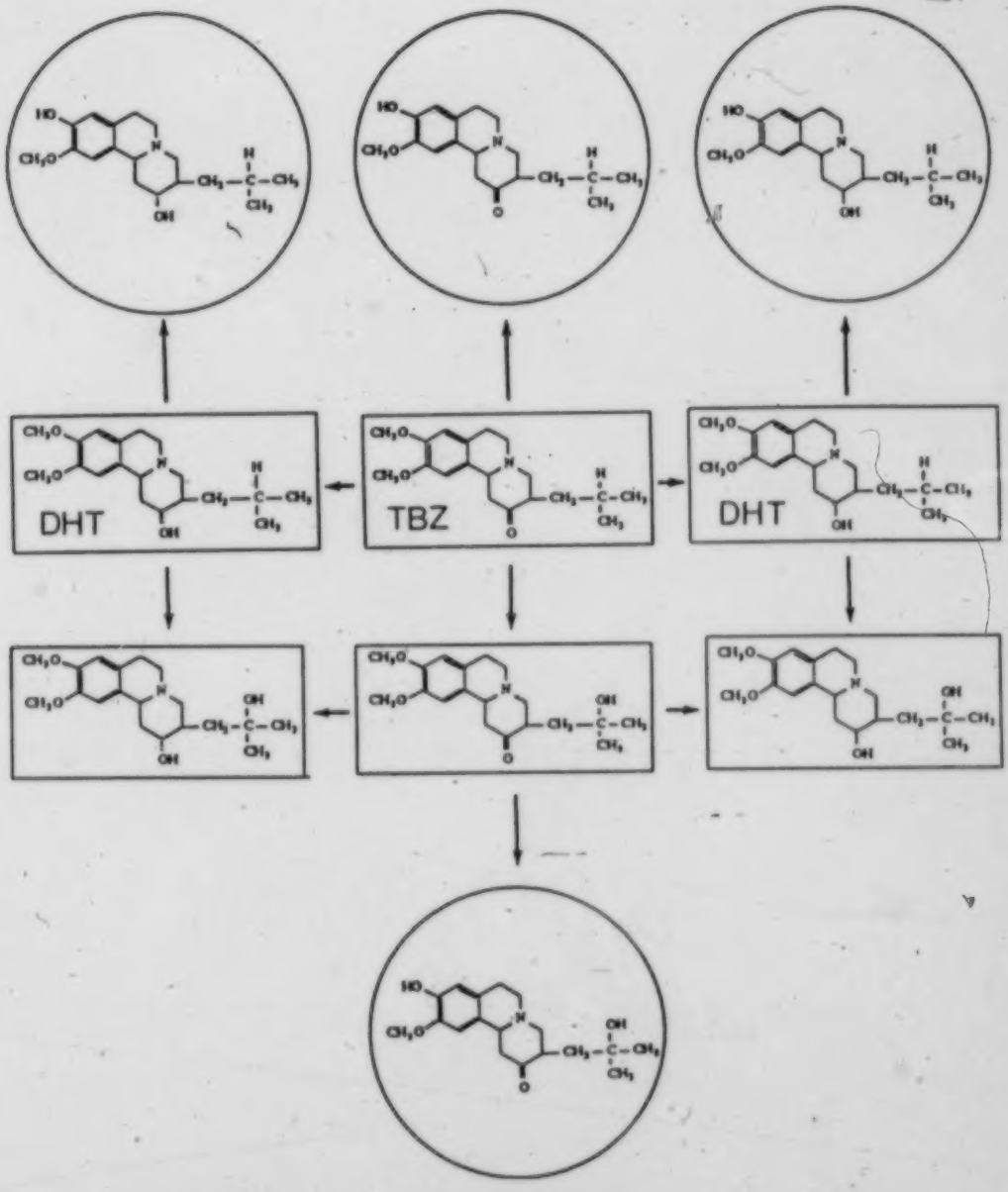


Fig. 1-2. Chemical structure and suggested metabolic pathways of TBZ (Adapted from the reference 26).
▭, unconjugated metabolite/drug; ◯, conjugated metabolite.

1.2. The Disease

1.2.1. History

Tardive dyskinesia (TD) refers to involuntary abnormal movements which usually appear in a proportion of patients exposed to long-term neuroleptic therapy (29-31). Although the disease is usually seen among patients with schizophrenia, it is not specific to that psychiatric diagnosis (32). The disorder has been recognized since the introduction of chlorpromazine into psychiatric practice in the 1950's (31). However, it is not yet clear whether neuroleptics really are its only cause. It has been noted by some investigators that a similar clinical pattern can also be found in patients never exposed to neuroleptics (33). Other terms (30) such as "buccolinguomasticatory syndrome" and "terminal extrapyramidal insufficiency syndrome" have also been utilized to describe the disease. Many patients who develop TD during chronic neuroleptic treatment recover when the offending drug is withdrawn. However, in some cases it may persist for years or even permanently, despite drug withdrawal (33).

1.2.2. Clinical Features

Tardive dyskinesia is characterized by involuntary hyperkinetic movements, varying in localization and appearance, occurring most often in the oral region (34). These consist of sucking and smacking movements of the lips, cheek puffing, lateral jaw movements and writhing, thrusting, and rolling or fly-catching movements of the tongue (35). Furthermore, choreo-athetoid movements of the head, body and limbs are seen. Similar to other forms of hyperkinesia, the

dyskinetic movements disappear during sleep (36). The pattern of movements in TD (34) varies from patient to patient. In younger patients it often consists of regular, relatively simple tongue protrusions with synchronus jaw movements. In older patients, the movements are more complex and irregular, the tongue rotating and licking in all directions, both in and out of the mouth. In some cases TD is complicated by difficulties in speech and swallowing, and irregular respiration. In extremely severe cases the movements may be painful and even life-threatening.

1.2.3. Prevalence and Risk Factors

Unfortunately, in the area of TD, methodological differences and shortcomings make successful integration of the available information somewhat difficult. Consequently reports of the prevalence of TD are very variable, values of 0.5 up to 60% being reported (29,30,32,37).

In terms of risk factors (30,34,37,38), age is one of the most important parameters. Irreversible TD is rare before the age of 40, but in patients above the age of 55 it occurs more frequently. Female sex is the second most frequently suggested risk factor, but most of the studies have not been able to confirm that (37). Dosage and duration of drug exposure, drug type, polypharmacy, organic brain dysfunction, convulsive therapies, previous extrapyramidal side effects, prior use of anticholinergic antiparkinsonism drugs, drug-free intervals and psychiatric diagnosis are among the risk factors which have widely been discussed in the literature (30,34,38).

1.2.4. Pathophysiologic Basis of Tardive Dyskinesia

Traditional neuroleptics block postsynaptic dopamine (DA) receptors, leading to an increased acetylcholine turnover (39). This can explain the appearance of parkinsonism in the patients treated with neuroleptics. During long-term neuroleptic treatment, however, the DA receptor blockade leads to a dopaminergic hypersensitivity, which can be shown by an increased behavioral response to DA agonists (39). This is perhaps due to some adaptive mechanisms. Until recent years it was believed that TD was only due to dopaminergic hypersensitivity, but this theory cannot fully explain the pathophysiology of TD.

The pathophysiology of TD is still unclear. It seems that TD is a heterogenous syndrome depending on a balance between several neurotransmitters including GABA in the brain (40).

1.2.4.1. Dopamine Receptors

The dopaminergic system has attracted much attention during the past few years because of its important role in diverse neurologic and psychiatric disorders including TD, parkinsonism and schizophrenia (41). The DA receptors are located in many different regions of the brain; the highest concentration is in the basal ganglia. Dopaminergic activity within the basal ganglia is essential for coordinated motion. Specific DA receptors are found on both postsynaptic and presynaptic terminals within the basal ganglia. The presynaptic receptors seem to act as "autoreceptors" for the system and have important physiologic functions. Stimulation of the autoreceptors causes a decrease in the synthesis and release of DA.

Stimulation of D-1 receptors, the first identified DA receptor, results in the formation of cyclic adenosine 5'-monophosphate. The D-2 receptors mediate psychomotor dopaminergic behaviors (rotation, locomotion, anti-parkinsonian action, psychomimetic action, emesis and stereotypy) and a prolactin-lowering action (42). Recently it has been shown that D-1 receptors are also involved in psychomotor behavior. The D-2 receptor is the site of action of antipsychotic and antiparkinsonism drugs.

1.2.4.2. Dopamine Mechanisms

Several clinical studies indicate that TD may be associated with a pathophysiologic state of relative dopaminergic overactivity (39). Consequently, TD worsens on withdrawal of the antipsychotic drugs (34,39).

Development of denervation or disuse supersensitivity of DA receptors (39) is one of the suggested mechanisms for functional overactivity of central dopaminergic mechanisms in TD. Neurones whose receptors have developed this denervation hypersensitivity may respond in an abnormal manner to any DA that reaches these receptors (39,43).

Animal studies indicate that prolonged administration of antipsychotic drugs or other agents antagonistic to DA, followed by their discontinuation, produces a behavioral state of increased responsiveness to DA agonists (44). This functional supersensitivity to DA persists only for several weeks (45). Hence, the usually prolonged and frequently irreversible course of the syndrome seems

inconsistent with a relatively transient, purely pharmacologic state of disuse supersensitivity (39).

The possibility that prolonged exposure to neuroleptics may permanently alter presynaptic mechanisms is worthy of consideration. Neuroleptic drugs by blocking the presynaptic DA receptors (autoreceptors) may increase the DA turnover which in turn might cause DA overactivity. In animal experiments, however, accelerated turnover of DA in response to neuroleptic drugs is reported to be only a transient effect (39).

1.2.4.3. Nondopaminergic Mechanisms

The idea of postsynaptic DA receptor supersensitivity was the dominant hypothesis for an explanation of the pathophysiology of TD until few years ago, but recently several investigators have questioned its validity (40,46-52). Several observations such as co-existence of TD and parkinsonism, cannot be explained by this theory (50). Some investigators suggest that postsynaptic receptor supersensitivity may be responsible only for certain subtypes, especially withdrawal-emergent dyskinesias (46).

1.2.4.3.1. GABA Dysfunction

It has been suggested that TD is the result of neuroleptic-induced destruction of a subpopulation of GABA-containing neurons in the striatum (40). Apparently, chronic administration of neuroleptics decreases the activity of glutamic acid decarboxylase, the enzyme responsible for synthesis of GABA from glutamic acid, in the globus pallidus and substantia nigra (40). Recently it has been

suggested that neuroleptics induce TD by initially altering DA receptor sensitivity in the striatum and thereafter reducing GABA system function in the substantia nigra and pars reticulata (51).

One of the most interesting hypotheses is that there are distinct types of DA receptors and GABA neurons (51). In relation to GABA neurons in striatum, one type is inhibited by DA, while another type is stimulated by DA (52). This suggests that neuroleptic drugs might be able to induce hyperkinetic movement disturbances including TD, and at the same time, parkinsonism. In most cases, parkinsonism and/or sedation may suppress the hyperkinetic movements at the initial stage of the treatment. Later, when tolerance has developed to parkinsonism and sedation, the hyperkinetic movements may become manifest (50).

1.2.4.3.2.Other Neurotransmitters

It has been suggested that there is a reciprocal DA-acetylcholine (ACh) balance in the basal ganglia (39). Clinical pharmacology indicates opposite effects of DA and ACh in various diseases related to the basal ganglia system, TD, parkinsonism, Huntington's chorea and possibly also schizophrenia. The blockade of DA neurotransmission by antipsychotic drugs thereby increases the turnover and release of ACh within the striatum (39).

Some researchers have proposed that neurotransmitters other than DA, ACh, and GABA may be primarily involved in the pathogenesis of TD. Jeste and Wyatt (46) suggest that the contribution of noradrenergic mechanisms is of great importance in the development of persistent TD. This, however, needs further evaluation.

1.2.5. Treatment of Tardive Dyskinesia

1.2.5.1. Dopaminergic Approaches

Based on the DA-hypersensitivity theory, DA-depleting agents may be useful in the treatment of TD. This is indeed true, as beneficial effects of TBZ in TD have been reported in numerous clinical investigations (11,14,16,18,19,53-56). The drug is now considered as the drug of choice in the control of movement disorders in TD (19). Reserpine is also a pre-synaptically acting DA-depleting agent which has been utilized in the treatment of TD (19). Tetrabenazine, however, offers certain advantages over RES (refer to the section 1.1.).

The treatment of hyperkinesias with DA antagonists is theoretically based upon the so-called DA hypothesis of movement disorders, i.e. a relative overactivity of DA in the basal ganglia in hyperkinetic syndromes and a relatively reduced DA activity in hypokinetic syndromes. It is an unquestionable fact that DA-antagonistic drugs have a beneficial effect upon hyperkinetic symptoms. However, they have no curative effect upon the disease and in some cases (at least hypothetically) may even worsen the symptoms in the long-term (57).

Recently tiapride, an atypical neuroleptic of the substituted benzamide class which selectively blocks D-2 dopaminergic receptors, was used to treat TD (58,59), but the results were not satisfactory.

There is a novel treatment approach to TD, which is based on the hypothesis that DA sensitivity can be down-regulated by temporarily increasing DA levels. In one study (60) gradually increasing doses of levodopa were used and the patients were kept at each dose

increment for evidence of adaptation. Following adaptation to a one-month course of treatment at the target dose, the levodopa was discontinued. It was noticed that dyskinetic movements were modestly exacerbated initially, but at the end, about half of the patients showed a marked improvement (60).

Low dose, short term carbidopa/levodopa therapy has been used to treat the simultaneous TD and parkinsonism with relatively successful results (61).

1.2.5.2.Non-dopaminergic Treatment Approaches

In 1979, the effectiveness of muscimol, an agonist of GABA, in the control of movements in TD was reported (62). The investigators, however, mentioned that this drug would not have practical value as a treatment agent, because of its behavioral side effects: mind altering properties and psychosis-worsening effects. Gamma-acetylenic GABA (GAG), an irreversible catalytic inhibitor of GABA-transaminase, was also tried for treatment of TD (63). Administration of this drug to patients significantly reduced TD. However, similar to muscimol, the side effects of GAG precluded it from clinical use.

The effect of valproate, an anticonvulsant drug which increases brain GABA levels, has been investigated in TD (64). The results, however, are contradictory.

Baclofen, a structural analogue of GABA, has also been reported to have no significant effect in TD (65). Progabide is a new GABA agonist which has been tried in the treatment of neuroleptic- and l-Dopa-induced dyskinesia (66). This drug appears to have a genuine

therapeutic activity in neuroleptic induced dyskinesia, but does not have any therapeutic effect on abnormal movements in l-Dopa-induced dyskinesia (66). Benzodiazepines may also enhance GABA function by potentiating GABA mechanisms (67). Diazepam has both reduced and aggravated TD. However, the question as to whether antihyperkinetic effects with benzodiazepines occur through specific or nonspecific sedative effects remains to be answered (67).

Augmenting cholinergic functions would be a logical therapeutic approach for TD. The rationale for increasing cholinergic influence is to counterbalance the relative excess of DA underlying TD. Cholinomimetic drugs like physostigmine, choline, and lecithin have shown variable effects complicated by undesirable side effects (67). In some cases, a slight antihyperkinetic effect was seen, but no effect or even aggravation of TD has been observed in other cases. RS 86, a new specific muscarinic agonist, produced no significant effects in TD either (68). It seems that treatment with cholinomimetics has no or only limited beneficial effect in TD.

Serotonergic agents have been postulated as potential therapeutic agents in TD. This is based on the serotonergic input to the basal ganglia and the clinical observations that tryptophan and pyridoxine caused a rapid deterioration in patients with Parkinson's disease. However, treatment with tryptophan and 5-hydroxytryptophan has not consistently reduced TD (67).

Ceruletide, a potent analogue of cholecystokinin octapeptide has also been tested in TD (69). Extremely severe and repetitious gross oral movements were observed within a few minutes after injection of

the drug and were continued for up to 1 h. Thereafter, oral movements tended to decrease gradually.

Adrenergic drugs, lithium, estrogens, pyridoxine, fusaric acid, manganese, barbiturates, phenytoin, alpha-tocopherol, and melanocyte-stimulating hormone release-inhibiting factor 1 have all been tried and produced mixed results in TD (67,70).

In conclusion, aside from TBZ, no drug treatments have been shown to be both safe and effective over extended treatment periods. The results obtained with TBZ, however, seem to be promising, as the drug is proven to be safe and the success rates are relatively high.

1.3. Hypothesis

There is a direct correlation between plasma concentrations of TBZ and/or its metabolite(s) and pharmacologic and consequently clinical effects of the drug.

1.4. Objectives

1. Development of a sensitive assay capable of measuring very low concentrations of TBZ and its major metabolite(s) in biological specimens.
2. Delineation of pharmacokinetics of the drug and its metabolite(s) in patients affected by TD.
3. Development and use of a suitable animal model to study pharmacokinetics of the drug and its metabolite(s).
4. Utilization of the animal model to study pharmacodynamics of the drug and its metabolite(s).

1.5. References

1. A. Brossi, H. Lindlar, M. Walter, and O. Schnider: Syntheseversuche in der Emetin-Reihe. 2-Oxohydrobenzo[a]chinolizine. Helv. Chim. Acta **41**, 119-139 (1958).

2. G. P. Quinn, P. A. Shore, and B. B. Brodie: Biochemical and Pharmacological Studies of RO 1-9569 (Tetrabenazine), a Non-Indole Tranquilizing Agent With Reserpine-Like Effects. J. Pharmacol. Exp. Ther. **127**, 103-109 (1959).

3. A. Pletscher, H. Besendorf, and K. F. Gey: Depression of Norepinephrine and 5-hydroxytryptamine in the Brain by Benzoquinolizine Derivatives. Science **129**, 844 (1959).

4. A. Pletscher, A. Brossi, and K. F. Gey: Benzoquinolizine Derivatives: A New Class of Monoamine Decreasing Drugs With Psychotropic Action. Int. Rev. Neurobiol. **4**, 275-306 (1962).

5. C. Onn-Leng and R. A. Webster: Effect of Tetrabenazine and α-methyl-m-tyrosine on Exploratory Activity and Brain Catecholamines in Rats. Br. J. Pharmacol. **41**, 691-699 (1971).

6. S. Ahlenius, J. Engel, H. Eriksson, and P. Sodersten: Effects of Tetrabenazine on Lordis Behaviour and on Brain Monoamines in the Female Rat. J. Neural Transm. **33**, 155-162 (1972).

7. A. Saner and A. Pletscher: A Benzo[a]quinolizine Derivative With a Neuroleptic-Like Action on Cerebral Monoamine Turnover. J. Pharmacol. Exp. Ther. **203**, 556-563 (1977).

8. T. Yohizaki and K. Tonda: Effects of Tricyclic Antidepressants on Tetrabenazine-Induced Depletion of Brain Monoamines in Rats. 1. Norepinephrine. Japan. J. Pharmacol. **30**, 721-729 (1980).

9. T. Yohizaki and K. Tonda: Effects of Tricyclic Antidepressants on Tetrabenazine-Induced Depletion of Brain Monoamines in Rats. 2. Dopamine. Japan. J. Pharmacol. **31**, 443-449 (1981).

10. D. J. Pettibone, J. A. Totaro, and A. B. Pflueger: Tetrabenazine-Induced Depletion of Brain Monoamines: Characterization and Interaction With Selected Antidepressants. Eur. J. Pharmacol. **102**, 425-430 (1984).

11. J. Jankovic: Treatment of Hyperkinetic Movement Disorders With Tetrabenazine: A Double-Blind Crossover Study. Ann. Neurol. **11**, 41-47 (1982).

12. O. Lingjaerde: Tetrabenazine (Nitoman) in the Treatment of Psychoses. Acta Psychiatr. Scand. suppl **170**, 11 (1963).

13. D. L. McLellan, R. J. Chalmers, and R. H. Johnson: A Double-Blind Trial of Tetrabenazine, Thiopropazate, and Placebo in Patients With Chorea. Lancet 1, 104-107 (1974).

14. E. Brandrup: Tetrabenzaine Treatment in Persisting Dyskinesia Caused by Psychopharmaca. Am. J. Psychiatry 118, 551-552 (1961).

15. M. M. Hoehen and N. Cherington: Spinal Myoclonus. Neurology 27, 942-946 (1977).

16. M. Swash, A. H. Roberts, H. Zakko, and K. W. G. Heathfield: Treatment of Involuntary Movement Disorders With Tetrabenazine. J. Neurol. Neurosurg. Psychiatry 35, 186-191 (1972).

17. J. Pearce: Reversal of Hemiballismus by Tetrabenazine. JAMA 219, 1345, (1972).

18. H. Pakkenberg: The Effects of Tetrabenzine in Some Hyperkinetic Syndromes. Acta Neurol. Scand. 44, 391-393 (1968).

19. S. Fahn: Long-Term Treatment of Tardive Dyskinesia With Presynaptically Acting Dopamine-Depleting Agents. Adv. Neurol. 7, 267-276 (1983).

20. A. Reches, R. E. Burke, C. M. Kuhn, M. N. Hassan, V. R. Jackson and S. Fahn: Tetrabenazine, an Amine-Depleting Drug, Also Blocks Dopamine Receptors in Rat Brain. J. Pharmacol. Exp. Ther. 225, 515-521 (1983).

21. Z. L. Kruk and C. J. Pycock: "Neurotransmitters and Drugs" Biddle Ltd., Surrey, England, 1983.

22. B. O. Mikkelsen: Tolerance of Tetrabenzine During Long-Term Treatment. Acta Neurol. Scand. 68, 57-60 (1983).

23. M. A. Dalby: Effect of Tetrabenzine on Extrapyramidal Movement Disorders. Br. Med. J. 2, 422-423 (1969).

24. D. W. Kidd and D. L. McLellan: Self-Poisoning With Tetrabenazine. Br. J. Clin. Pract. 26, 179-180 (1972).

25. O. Lingjaerde: Tetrabenzine in The Treatment of Psychoses. Acta Psychiatr. Scand. Suppl. 170, 16 (1963).

26. D. E. Schwartz, H. Bruderer, J. Rieder and A. Brossi: Metabolic Studies of Tetrabenzine, a Psychotropic Drug in Animals and Man. Biochem. Pharmacol. 15, 645-655 (1966).

27. H. Maurer and K. Pfleger: Screening Procedure for Detection of Phenothiazine and Analogous Neuroleptics and Their Metabolites in Urine Using a Computerized Gas Chromatographic-Mass Spectrometric Technique. J. Chromatogr. 306, 125-145 (1984).

28. M. S. Roberts, S. McLean, K. S. Millingen, and H. M. Galloway: The Pharmacokinetics of Tetrabenzine and Its Hydroxy Metabolite in Patients Treated for Involuntary Movement Disorders. Eur. J. Clin. Pharmacol. 29, 703-708 (1986).

29. R. C. Chacko, L. Root, J. Marmion, V. Molinari, and G. L. Adams: The Prevalence of Tardive Dyskinesia in Geropsychiatric Outpatients. J. Clin. Psychiatry 46, 55-57, (1985).

30. J. M. Kane, and J. M. Smith: Tardive Dyskinesia, Prevalence and Risk Factors, 1959 to 1979. Arch. Gen. Psychiatry 39, 473-481 (1982).

31. H. L. Klawans and W. J. Weiner "Textbook of Clinical Neuropharmacology" Raven Press, 1981.

32. D. G. Owens, E. C. Johnstone, and C. D. Frith: Spontaneous Involuntary Disorders of Movement. Arch. Gen. Psychiatry 39, 452-461 (1982).

33. C. D. Marsden: Is Tardive Dyskinesia a Unique Disorder? In "Dyskinesia, Research and Treatment" (D. E. Casey, T. N. Chase, A. V. Christensen, and J. Gerlach, eds.) pp. 64-71. Springer-Verlage, N.Y. (1985).

34. J. Gerlach: Tardive Dyskinesia. Dan. Med. Bull. 26,209-245 (1979).

35. R. H. Lenox, L. A. Weaver, and B. M. Saran: Tardive Dyskinesia: Clinical and Neuroendocrine Response to Low Dose Bromocriptine. J. Clin. Psychopharmacol. 5, 286-292 (1985).

36. Editorial: Neurologic Sympyoms Associated With Antipsychotic Drug Use. N. Engl. J. Med. 289, 20-23 (1973).

37. S. Mukherjee, A. M. Rosen, C. Cardenas, V. Varia, and S. Olarte: Tardive Dyskinesia in Psychiatric Outpatients. Arch. Gen. Psychiatry 39, 466-469 (1982).

38. J. M. Kane, M. Woerner, and J. Lieberman: Tardive Dyskinesia: Prevalence, Incidence, and Risk Factors. In "Dyskinesia, Research and Treatment" (D. E. Casey, T. N. Chase, A. V. Christensen, and J. Gerlach, eds.) pp. 72-78. Springer-Verlage, N.Y. (1985).

39. D. Tarsy, and R. J. Baldessarini: The Pathophysiologic Basis of Tardive Dyskinesia. Biol. Psychiatry 12, 431-450 (1977).

40. H. C. Fibiger and K. G. Lloyd: Neurobiological Substrates of Tardive Dyskinesia : The GABA Hypothesis. Trends NeuroSci. 7, 462-464 (1984).

41. H. N. Wagner: Imaging CNS Receptors: The Dopaminergic System. Hosp. Pract. 19, 187-202 (1984).

42 P. Seeman: Brain Dopamine Receptors. Pharmacol. Rev. 32, 229-313 (1980).

43. H. L. Klawans: The Pharmacology of Tardive Dyskinesia. Am. J. Psychiatry **130**, 82-86 (1973).

44. P. F. Vonvoigtlander, E. G. Losey, and H. J. Triezenberg: Induced Sensitivity to Dopaminergic Agents After Chronic Neuroleptic Treatment. J. Pharmacol. Exp. Ther. **193**, 88-94 (1975).

45. D. Tarsy, and R. J. Baldessarini: Behavioral Supersensitivity to Apomorphine Following Chronic Treatment With Drugs Which Interfere With The Synaptic Function of Catecholamines. Neuropharmacology **13** , 927-940 (1974).

46. D. V. Jeste, and R. J. Wyatt: Dogma Disputed: Is Tardive Dyskinesia Due to Postsynaptic Dopamine Receptor Supersensitivity? J. Clin. Psychiatry **42**, 455-457 (1981).

47. J. L. Waddington, A. J. Cross, S. Gamble, and R. C. Bourne: Spontaneous Orofacial Dyskinesia and Dopaminergic Function in Rats After 6 Months of Neuroleptic Treatment. Science 220, 530-532 (1983).

48. F. Muller-Spahn F., M. Ackenheil, M. Albus, G. May, D. Naber, D. Welter, and K. Zander: Neuroendocrine Effects of Apomorphine in Chronic Schizophernic Patients Under Long-Term Neuroleptic Therapy and After Drug Withdrawl: Relations to Psychopathology and Tardive Dyskinesia. Psychopharmacology **84**, 436-440 (1984).

49. J. L. Waddington: Further Anomalies in The Dopamine Receptor Supersensitivity Hypothesis of Tardive Dyskinesia. Trends NeuroSci **8**, 200 (1985).

50. J. Gerlach: Pathophysiological Mechanisms Underlying Tardive Dyskinesia. In "Dyskinesia, Research and Treatment" (D. E. Casey, T. N. Chase, A. V. Christensen, and J. Gerlach, eds) pp. 98-103. Springer-Verlage, N.Y. (1985).

51. C. A. Tamminga, G. K. Thaker, and T. N. Chase: GABA Dysfuction in Pathophysiology of Tardive Dyskinesia. In "Dyskinesia, Research and Treatment" (D. E. Casey, T. N. Chase, A. V. Christensen, and J. Gerlach, eds.) pp. 122-127. Springer-Verlage, N.Y. (1985).

52. J. Scheel-Kruger, and J. Arnte: New Aspects on The Role of Dopamine, Acetylcholine, and GABA in The Development of Tardive Dyskinesia. In "Dyskinesia, Research and Treatment" (D. E. Casey, T. N. Chase, A. V. Christensen, and J. Gerlach, eds.) pp. 46-57. springer-Verlage, N.Y. (1985).

53. D. Kingston: Tetrabenazine for Involuntary Movement Disorders. Med. J. Aust. **1**, 628-630 (1979).

54. S. W. Asher and M. J. Aminoff: Tetrabenazine and Movement Disorders. Neurology 31, 1051-1054 (1981).

55. H. Kazamatsuri, C. P. Chien, and J. O. Cole: Long-Term Treatment of Tardive Dyskinesia With Haloperidol and Tetrabenazine. Am. J. Psychiatry 130, 479-483 (1973).

56. M. Bartels and E. Zoller: Tetrabenazine (Nitoman) Therapy of Chronic Spontaneous Oral Dyskinesia. Eur. Arch. Psychiatry Neurol. Sci. 234, 172-174 (1984).

57. R. Fog: The Effect of Dopamine Antagonists in Spontaneous and Tardive Dyskinesia. In "Dyskinesia, Research and Treatment" (D. E. Casey, T. N. Chase, A. V. Christensen, and J. Gerlach, eds.) pp. 118-121. Springer-Verlage, N.Y. (1985).

58. P. Pollak, J. M. Gaio, M. Hommel, J. Pellat, and J. Perret: Effects of Tiapride in Tardive Dyskinesia. Psychopharmacology 85, 236-239 (1985).

59. W. Greil, S. Auberger, H. Hoag, and E. Ruther: Tiapride: Effects on Tardive Dyskinesia and on Prolactin Plasma Concentrations. Neuropsychobiology 14, 17-22 (1985).

60. M. Alpert, A. J. Friedhoff, and F. Diamond F: Use of Dopamine Receptor Agonists to Reduce Dopamine Receptor Number as Treatment for Tardive Dyskinesia. Adv. Neurol. 37, 253-258 (1983).

61. I. Shoulson: Carbidopa/levodopa Therapy of Coexistent Drug Induced Parkinsonism and Tardive Dyskinesia. Adv. Neurol. 37, 259-266 (1983).

62. C. A. Tamminga, J. W. Crayton, and T. N. Chase: Improvement in Tardive Dyskinesia After Muscimol Therapy. Arch. Gen. Psychiatry 36, 595-598 (1979).

63. D. E. Casey, J. Gerlach, G. Mayelund, and T. R. Christensen: Gama-Acetylenic GABA in Tardive Dyskinesia. Arch. Gen. Psychiatry 37, 1376-1379 (1980).

64. H. A. Nasrallah, and C. W. Mc: A Placebo-Controlled Trial of Valproate in Tardive Dyskinesia. Biol. Psychiatry 20, 205-208 (1985).

65. W. M. Glazer, D. C. Moore, M. B. Bowers, B. S. Bunny, and M. Roffman: The Treatment of Tardive Dyskinesia With Baclofen. Psychopharmacology 87, 480-483 (1985).

66. P. L. Morselli, V. Fournier, L. Bossi, and B. Musch: Clinical Activity of GABA Agonists in Neuroleptic- and L-Dopa-Induced Dyskinesias. In "Dyskinesia, research and treatment" (D. E. Casey, T. N. Chase, A. V. Christensen, and J. Gerlach, eds.) pp. 128-136. Springer-Verlage, N.Y. (1985)

67. D. E. Casey: Tardive dyskinesia: Nondopaminergic Treatment Approaches. In "Dyskinesia, Research and Treatment" (D. E. Casey, T. N. Chase , A. V. Christensen, and J. Gerlach, eds.)pp. 137-144. Springer-Verlage, N.Y. (1985).

68. U. Noring, U. J. Povlsen, D. E. Casey, and J. Gerlach: Effect of a Cholinomimetic Drug (RS 86) in Tardive Dyskinesia and Drug-Related Parkinsonism. Psychopharmacology **84**, 569-571 (1984).

69. T. Nishikawa, M. Tanaka, I. Koga, and Y. Uchida: Biphasic and Long-Lasting Effect of Ceruletide on Tardive Dyskinesia. Psychopharmacology **86**, 43-44 (1985).

70. J. B. Lohr, J. L. Cadet, M. A. Lohr, D. V. Jeste, and R. J. Wyatt: Alpha-Tocopherol in Tardive Dyskinesia. Lancet 1, 913-914 (1987).

2. DIRECT INJECTION HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF TETRABENAZINE AND ITS METABOLITES IN PLASMA OF HUMANS AND RAT*

2.1. Introduction

Tetrabenazine (1,3,4,6,7,11b-hexahydro-3-isobutyl-9,10-dimethoxy-2H-benzo(a)quinolizine-2-one; TBZ) is a synthetic benzoquinolizine derivative which, like reserpine, depletes monoamines in the central nervous system (1). Originally introduced as an antipsychotic agent in 1958 (2), the drug failed to elicit potent antipsychotic properties and was consequently withdrawn from the market. Recently, however, its effect in controlling some movement disorders has attracted considerable attention (3-6). It appears to be a drug of choice in the treatment of tardive dyskinesia (TD) (5) and Huntington's chorea (6). However, the treatment with TBZ is empirical as its pharmacokinetics are unknown. This is mainly due to the unavailability of a suitable assay to determine the drug and its metabolites in biological samples. A previously reported HPLC method (7) involved a lengthy extraction procedure and since an internal standard (IS) was not used, it appeared to be subject to considerable experimental error.

The objective of this chapter is to report a sensitive, selective and convenient HPLC assay of TBZ and one of its major metabolites, dihydrotetrabenazine (DHT) in plasma of humans and rats.

---

* A version of this chapter has been published:
R. Mehvar, F. Jamali, M. W. B. Watson, and D. Skelton. J. Pharm. Sci. **75**, 1006-1009 (1986).

2.2. Experimental Section

2.2.1. Materials

Tetrabenazine (lot 61-84) and dihydro metabolite (lot 39-85) were gifts from Hoffmann-LaRoche of Canada (Etobicoke, Ont.). Internal standards (IS) were 5,6-benzoquinoline (B.D.H. Laboratory, England) and diazepam (Sigma Chem. Co., St. Louis, MO). Acetonitrile and water were HPLC grade, while triethylamine (TEA), acetic acid, ethyl acetate, sodium acetate and mercuric acetate were analytical reagent grade.

2.2.2. Apparatus

A 590 solvent pump was attached to a Wisp 710-B autosampler (Waters, Mississauga, Ont.) and a GM 970 fluorometer (Kratos, Westwood, NJ). The detector was set at excitation and emission wavelengths of 265 and 418 nm, respectively. The chromatograms were recorded on a 3390A integrator (Hewlett-Packard, Mississauga, Ont.). The UV absorbance of TBZ and DHT were measured using a variable-wavelength detector (model 481, Waters, Mississauga, Ont.) at 280 nm.

Mass spectra (electron impact and chemical ionization) were obtained on a Varian 6000 GL/VG 7070E MS/POP 11/24 data system (AnalyTech Instrumentation and Service, St. Laurent, Canada). For CI spectra, ammonia was used as the reactant gas.

2.2.3. Standard Solution

For the fluorescence method, a 3% (w/v) solution of mercuric acetate in water containing 5% acetic acid and 5% sodium acetate was

prepared. Stock solutions of TBZ and DHT were prepared by dissolving the compounds in approximately 2 mL of methanol and then adding water to a final volume of 100 mL. Blank plasma was spiked with standard solutions of the compounds prepared by diluting the stock solution to contain final concentrations of 0.1, 0.5, 1, 2, 5, 10, 20, 50, 100 and 200 ng/mL for tetrabenzine and dihydrotetrabenazine, and 500 and 1000 ng/mL for dihydrotetrabenazine. To examine the effect of plasma on the fluorescence intensity of the compounds, similar solutions with final concentrations of 0.5, 1, 5, 20 and 100 ng/mL of the tetrabenazine and 5, 20 and 100 ng/mL of dihydrotetrabenazine were prepared in duplicate in the absence of plasma. The IS solution contained 50 ng/mL of 5,6-benzoquinoline.

For UV detection, stock solutions of TBZ and DHT were prepared in 5 mM hydrochloric acid. Blank plasma was spiked with the stock solution to produce final concentrations of 0.5, 1.0, 2.5, 5.0 and 10.0 mg/L of the drug and its metabolite. The IS solution was prepared by dissolving 10 mg diazepam in 200 mL methanol.

Calibration curves were constructed by plotting the peak area ratio of the compounds to IS vs plasma TBZ and DHT concentrations.

2.2.4. Sample Preparation

For the fluorescence assay, 0.4 mL of the mercuric acetate solution was combined with 0.4 mL of plasma in a microcentrifuge tube. The tubes were vortexed for 30 sec, centrifuged (Microcentrifuge model 235A, Fisher, Edmonton, AB) for 5 min and 0.6 mL of the supernatant was transferred to glass tubes. The tubes were then heated at 110^{o}C for 1 h. After cooling to ambient

temperature, 0.1 mL of IS solution was added and the contents of the tubes were transferred to autosampler vials. Depending on the concentration, aliquots of 0.025 to 0.1 mL were then injected into the instrument.

For the UV assay, 1 mL of plasma was combined with 0.05 mL of IS solution, 0.1 mL of a 0.2 M solution of sodium hydroxide, and 4 mL of ethyl acetate. The tubes were vortexed for 30 sec and centrifuged (Adams Dynac, USA high-speed centrifuge) for 3 min at 5,000 rpm. The organic layer was evaporated using a Savant Speed Vac concentrator/evaporator (Emerston Instrument, Scarborough, Canada) and the residue dissolved in 0.2 mL of acetonitrile. Aliquots of 0.01 to 0.025 mL of the reconstituted solutions were injected into the HPLC. To estimate the extraction efficiency of this method, blank plasma samples were spiked with IS and extracted as described above. After evaporation, the contents of the tubes were dissolved in 0.2 mL of acetonitrile containing tetrabenazine and its metabolites to give final concentrations corresponding to those of 1 and 5 mg/L in plasma (n = 2). Aliquots of 0.02 mL of the solutions were then injected into HPLC and the responses were compared with those observed following extraction from solutions of equal concentrations.

2.2.5. Chromatography

Resolution of TBZ and DHT and their dehydro derivatives, and at least three unknown metabolites, was achieved using a 4.6 mm x 10 cm analytical column containing 5 micron octadecylsilane packing material (Partisil 5 ODS-3, Whatman, Clifton, NJ) at 60^{o}C (model

1122/WTC-120 oven, Waters, Mississauga, Ont.) for the fluorescence-HPLC and at ambient temperature for the UV-HPLC with a precolumn module (Guard-Pak with uBondapak C18 end-capped inserts, Waters, Mississauga, Ont). Other reversed-phase columns tested for their performance were 3.9 mm x 30 cm stainless steel uBondapak C18, 8 mm x 10 cm Novapak C18 Radial-Pak and 8 mm x 10 cm uBondapak C18 Radial-Pak from Waters (Mississauga, Ont.); 4.16 mm x 12.5 cm Partisphere cartridge (Whatman, Clifton, NJ); and 4.6 mm x 25 cm stainless steel RP-18 (Serva, Heidelberg, FRG).

For the fluorescence assay, the mobile phase consisted of water:acetonitrile:acetic acid:TEA (65:33:2:0.15). The flow-rate was set at 0.6 mL/min. The UV analysis was performed using a solution of water:acetonitrile:TEA:acetic acid (50:50:0.2:0.1) as the mobile phase. The flow-rate and column were the same as those described for the fluorescence method. The pH of the aqueous component of the mobile phases were 3.8 and 6.2 for the fluorescence and UV assays, respectively.

2.2.6. Patient Study

Two patients affected by TD whose movements were controlled by TBZ, and a healthy subject, volunteered to participate in the project. The objectives and implications of the experiment were explained to them and signed informed consent forms were obtained. Patient EP was a 42-year-old, 120 kg man who was taking 25 mg of the drug tid. He was also receiving chlorpromazine (100 mg, tid) and diazepam (5 mg, tid), both at 6:00, 14:00 and 20:00 h. Patient MM was a 62-year-old, 75 kg woman who was under treatment with 37.5 mg

of tetrabenazine tid. She was also taking acetaminophen 650 mg, qid. Both patients appeared to have normal kidney and liver functions. The drug (Nitoman tablets, Hoffmann-LaRoche, Etobicoke, Ont.) was administered at 8:00, 13:00 and 18:00 h. Blood samples (3 mL) were taken just before and then 0.5, 1.0, 2.0, 3.0 and 5.0 h after the first and second dose and 0.5, 1.0, 2.0, 3.0 and 14.0 h after the last dose. The healthy volunteer was a 39-year-old man who took a single oral dose of 25 mg of the drug and donated blood samples just before and 1.5 h after the dose. He did not take any other medications two weeks before and during the experiment. Plasma was separated and kept frozen at -20°C until analyzed.

2.2.7. Animal Study

Tetrabenazine (1 mg/kg, dissolved in 1mM HCl) was injected into the jugular vein of four male Sprague-Dawley rats, weighing 250 - 300 g, through a previously inserted catheter. Blood samples (0.25 mL) were taken at 0, 5 and 30 min, and at 2, 4, 8, 12 and 23 h, and transferred into microcentrifuge tubes containing sodium citrate. Blood plasma was separated and assayed for TBZ and DHT as described above.

2.2.8. Validation Test

Aliquots of patient MM's plasma samples were pooled (total of 3 mL) and assayed for TBZ and DHT before (UV detection) and after (fluorescence detection) oxidation.

Under light anesthesia, single doses of the drug (1 - 5 mg/kg) were injected through the jugular vein into five Sprague-Dawley male

rats weighing 250-300 g. Approximately 10 min after administration, 3-10 mL of blood were collected in the presence of sodium citrate as anticoagulant. Plasma was separated and kept at -20°C until analysis. The specimens were assayed using both UV detection for unchanged TBZ and DHT, and fluorescence detection, for oxidized TBZ and DHT. The estimated concentrations were then compared.

The identities of TBZ and DHT in biological samples were confirmed by comparing the electron impact and chemical ionization GC-MS properties of the peaks separated from UV-HPLC eluants with those of authentic standards.

2.3. Results and Discussion

An advantage of reversed-phase chromatography is that the biological samples can be directly injected into the HPLC provided that the detector possesses sufficient sensitivity. We have previously reported application of the direct-injection method in analyzing non-steroidal antiinflammatory drugs (NSAID) (8). The therapeutic plasma concentrations of NSAIDs are usually in the range of mg/L, thus their quantification is conveniently achieved using ultraviolet detectors. The plasma concentrations of TBZ following administration of the recommended doses, on the other hand, seem to be in the range of ng/mL, which is below the normal range of existing UV detectors. Fluorescence detection seems to be an appropriate alternative. However, neither the drug nor its known metabolite possesses strong fluorogenic properties. Schwartz and his co-workers (9,10) used TLC to separate TBZ and its metabolites. They then visualized the resolved compounds by spraying with a solution of

mercuric acetate and heating the plates at 110°C for 10 min. Under these conditions, benzoquinolizine derivatives are expected to be oxidized to highly fluorescent derivatives. Leonard et al. (11), who used mercuric acetate to dehydrogenate the bicyclic tertiary amine, quinolizidine, identified the product as $\Delta^{1(10)}$-dehydroquinolizidine. A similar dehydrogenated product for tetrabenazine, a tricyclic tertiary amine, is feasible. Roberts et al. (7) utilized this reaction for the HPLC analysis of TBZ and DHT in plasma extracts. However, their method (7) had two main shortcomings: it involved a lengthy extraction procedure and was carried out in the absence of an IS. According to Roberts et al. (7), this method could measure approximately 0.1 ng/mL of TBZ and 1.0 ng/mL of DHT in plasma; in our hands, TBZ and DHT could not be determined when their respective concentrations were less than 10 and 50 ng/mL. However, when the extraction process was eliminated from the sample preparation procedure, the detection limit was improved severalfold. It is, therefore, reasonable to suggest that the extraction method used by Roberts et al. (7) may have a poor efficiency. Using the direct-injection method reported here, the minimum quantifiable concentrations for TBZ and DHT were found to be 0.5 and 2.0 ng/mL, respectively. The signal:noise ratios for 0.5 and 2.5 ng/mL of TBZ and DHT in plasma preparations were >6 and >8, respectively. Concentrations of <0.5 and <2.0 ng/mL of TBZ and DHT, however, were associated with coefficient of variation of >10%.

Various reagents including acetonitrile, trichloroacetic acid and zinc sulfate were used to precipitate plasma proteins. The most satisfactory results were obtained following the addition of mercuric

acetate which is also required for the oxidation of TBZ and DHT. At least 400 injections were made into the same column before a significant reduction in its efficiency was noticed.

To determine the time required to complete the dehydrogenation, TBZ and DHT were heated in the presence of mercuric acetate for 2 h at 110°C. The fluorescence intensity of the solutions were measured at 15-min intervals. At 110°C, the optimum time was found to be 45 min for TBZ and 1 h for DHT. Since the intensity remained constant after 1 h, this heating time was chosen for the purpose of oxidizing TBZ and DHT.

Figure 2-1 depicts chromatograms of plasma samples from a healthy subject before, and 1.5 h after, administration of 25 mg of TBZ and from patient MM just prior to the first routine daily dose. Retention times for TBZ and DHT were 7.5 and 5.5 min, respectively. For the healthy subject, three additional plasma components, presumably other metabolites of TBZ, were observed at 3.1, 3.6 and 4.4 min. In their HPLC trace, Roberts et al. (7) detected only one peak, in addition to TBZ and DHT and suggested that it might represent co-eluting metabolites of TBZ. This can be attributed to the deficiency of the column used by these authors as we also noticed that with the exception of Partisil 5 ODS-3, all of the tested columns exhibited poor peak resolution properties.

Plasma of the patients contained an extra peak in addition to the ones found in the specimen collected from the healthy subject. This peak eluted 2.6 min after injection. The extra peak can be attributed either to the accumulation of a metabolite or a group of metabolites eluted together, when TBZ is given repeatedly, or to the

Fig. 2-1. Chromatograms of plasma of a healthy subject before (a) and 1.5 h after (b) 25 mg tetrabenazine and that of a patient (c) taking 37.5 mg tetrabenazine tid for 8 months just before the first daily dose. Key: 1, 2 and 3 are tetrabenazine, its dihydro metabolite and internal standard (5,6-benzoquinoline), respectively. At detector sensitivity of 1.0, the recorder attenuation maintained at 2 except from 7 to 9 min which was changed to 0.

presence of other drugs concomitantly taken by the patients. However, as the nature of these additional drugs, i.e., acetaminophen in one case and diazepam and chlorpromazine in another, are so different from one another, it is unlikely that they appear in plasma as fluorogenic compounds with the same retention time. The extra peak, therefore, appears to be resulted from administrations of TBZ.

Figure 2-2 depicts chromatograms of rat plasma before, and 10 min after, intravenous administration of 1 mg/kg of tetrabenazine. The chromatograms of plasma samples from humans and rats are similar with respect to the number and retention times of observed peaks.

Reversed-phase HPLC of nitrogen containing compounds may result in broad asymmetric peaks (12,13). This has been attributed to the adsorption of these compounds to the free residual silanol groups on the silica gel column packing. Peaks representing dehydrogenated TBZ and DHT were broad and showed considerable tailing in the absence of TEA. Furthermore, without TEA, the relationship between the peak area ratios and the corresponding concentrations was non-linear for both TBZ and DHT, indicating considerable adsorption to the column (Fig. 2-3). The nonlinearity was particularly evident when Novapak (Radial-Pak) columns were used. The extent of adsorption was greater for DHT as compared to TBZ. Adsorption problems were eliminated by addition of TEA and increasing the column temperature to 60^{o}C (Fig. 2-3). Among the compounds examined for their suitability as IS (Table 2-1), the aromatic compound 5,6-benzoquinoline was selected because it showed favorable fluorogenic properties and was completely resolved from other peaks.

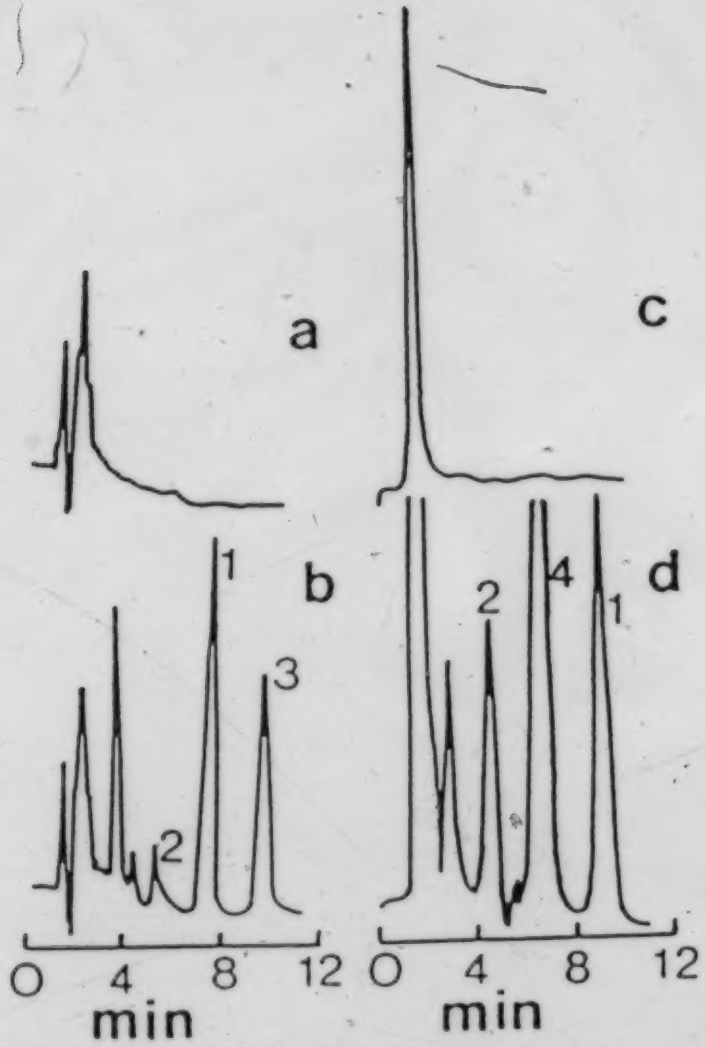


Fig. 2-2. Fluorescence- (a and b) and UV- (c and d) HPLCs of the rat plasma before (a and c) and 2 h (b) and 10 min (d) after 1 mg/kg and 5 mg/kg iv doses of the drug, respectively. Key: 1 and 2 represent tetrabenazine and the dihyrdo metabolite, and 3 (5,6-benzoquinoline) and 4 (diazepam) are ISs peaks.

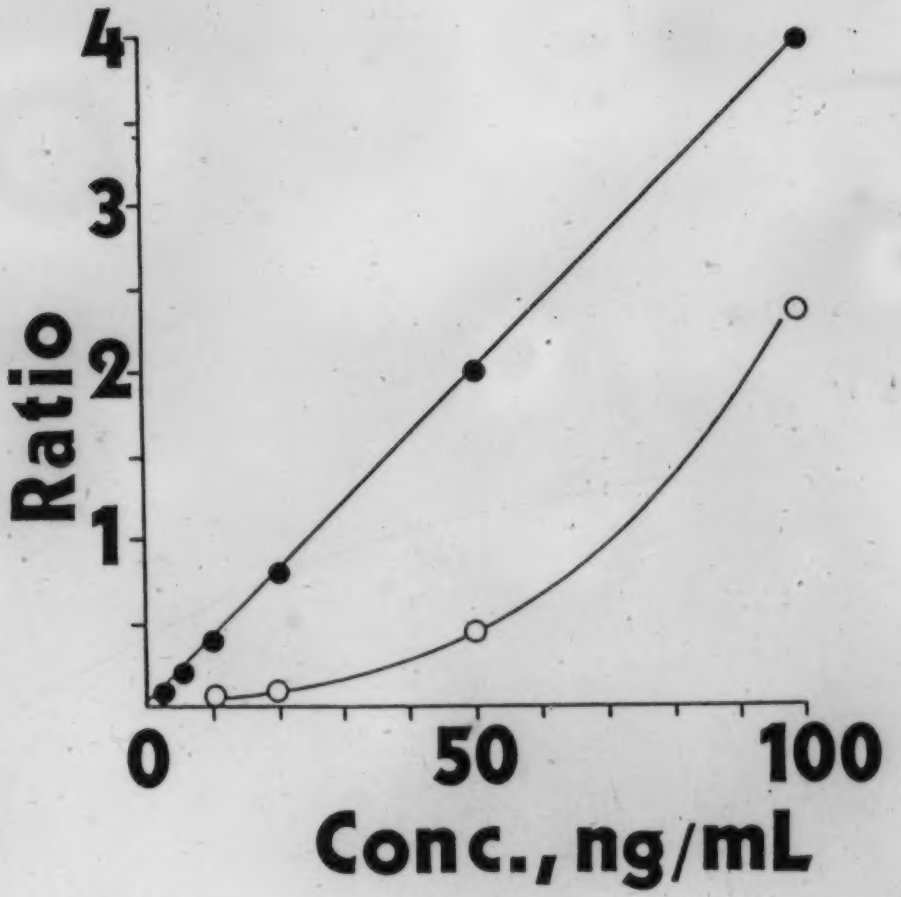


Fig. 2-3. Relationship between TBZ/IS area ratios and corresponding Plasma concentrations before (open circles) and after (closed circles) addition of triethylamine to the mobile phase.

Table 2-1. Compounds tested for their appropriateness as internal standard. Positive and negative signs indicate the presence and absence of the properties, respectively.

Compound	Dehydrogenation	Thermostability	Fluorogenity	Appropriateness of Retention time
5:6 Benzoquinoline[a]	-	+	+	+
Berberine HCl[b]	-	-	+	+
Coralyne Cl[b]	-	-	+	+
Corynanthine HCl[b]	+	-	+	-
Emetine HCl[b]	+	-	+	-
Etodolac[c]	-	-	+	+
Flurene[b]	-	-	-	+
Naproxen[d]	-	-	+	-
P-amino salicylic cid[b]	-	-	+	-
Phenylphenol[e]	-	-	+	+
Protriptylline[f]	-	-	+	+
Quinaldic acid[g]	-	-	+	-
Quinidine HCl[g]	-	-	+	-
Reserpine[g]	+	-	+	+
Sulfanilamide[b]	-	-	+	-
Yohimbine HCl[g]	+	+	+	-

Suppliers: a, BDH (England); b, Aldrich (Milwaukee, WI, USA); c, Ayerst (Montreal, PQ, Canada); d, Syntex (Palo Alto, CA, USA); e, Eastman Kodak (Rochester, NY, USA); f, MSD (Kirkland, PQ, Canada); g, Sigma (St. Louis, MO, USA).

Excellent linearity was found between the peak area ratios (TBZ/IS and DHT/IS) and plasma concentrations within the ranges of 0.5-200 and 2-1000 ng/mL for TBZ and DHT, respectively. The best-fit lines passing through the experimental points were $y = 0.0013 + 0.0392x$ and $y = 0.0038 + 0.0053x$ with correlation coefficents of 0.999 and 0.998 for TBZ and DHT, respectively. Within the examined range, the intraday CVs were within 1.9 to 6.3% for TBZ and 2.2 to 6.5% for DHT. The interday CVs were 2.8 to 7.9% and 2.5 to 8.0% for TBZ and DHT, respectively. The differences between the concentrations added to plasma and those found ranged from 0.1 to 9.9% within the examined range (Table 2-2). The recovery of the compounds from spiked plasma samples was approximately 100% (94.3-105% for TBZ and 97.4-104% for DHT).

To examine if the oxidation products were indeed derived only from the drug and its metabolite, we developed a UV-HPLC method which enabled us to measure TBZ and DHT in plasma samples in concentrations as low as 0.5 mg/L (Fig. 2-2). Within the range of 0.5-10 mg/L, the relationship between the concentrations and peak area (TBZ or DHT:IS) was linear (coefficient of correlation >0.991) with acceptable reproducibility (CV <8%, n = 3). The efficiency of the extraction method was between 92.0 - 97.0% and 100 - 101% for TBZ and DHT, respectively. Plasma concentrations of the drug and its metabolite, following intravenous administration of 1-5 mg/kg to rats were then measured using both UV- and fluorescence-HPLC (Table 2-3). The observed differences and disproportionalities in the plasma concentrations reported in Table 2-3 cannot be attributed only to the interanimal variations. As blood samples were not collected at

Table 2-2. Accuracy and Precision of the fluorescence-HPLC assay performed on spiked plasma samples[a]

Theoretical Conc., ng/mL	Measured Conc., ng/mL			
	Tetrabenazine	%Error	Dihydrotetrabenazine	%Error
0.500	0.490(7.9)	-2.0	-	-
1.00	1.08 (4.9)	8.0	-	-
2.00	2.17 (7.8)	8.5	1.88(5.1)	-6.0
5.00	4.97 (4.9)	-6.0	4.78(6.7)	-4.4
10.0	9.99 (3.9)	-0.1	9.01(8.0)	-9.9
20.0	19.7 (2.8)	-1.7	18.8 (3.8)	-5.9
50.0	48.1 (5.2)	-3.7	51.5 (2.6)	3.1
100	102 (5.6)	2.4	104 (3.6)	4.5
200	199 (2.9)	-0.3	196 (4.8)	-1.8
500	-	-	511 (3.1)	2.1
1000	-	-	1001 (2.5)	0.1

[a]Values in Parentheses are % CV; n = 4.

Table 2-3. Rat plasma concentrations (mg/L) of tetrabenazine and dihydrotetrabenazine approximately 10 min after intravenous administration of the drug and measurement with UV- and fluorescence-HPLC.

	Dose	Tetrabenazine		Dihydrotetrabenazine	
Rat	mg/kg	UV	Fluorescence	UV	Fluorescence
1	1.0	0.288	0.279	0.506	0.431
2	5.0	5.32	5.00	0.585	0.606
3	3.0	3.06	2.83	1.13	1.33
4	1.0	0.777	0.895	0.981	0.934
5	1.0	1.07	1.02	1.04	1.14

precisely the same time and the drug concentration declined very rapidly during the first few minutes postdosing (Fig. 2-4), the variations might, at least partly, be attributed to the different sampling time. The values estimated before (UV) and after (fluorescence) oxidation were similar (two-way ANOVA at $\alpha = 0.05$). This indicates that, indeed, the oxidized derivatives are derived only from TBZ and DHT. As the therapeutic plasma concentration of the drug is below the minimum quantifiable concentration for UV-HPLC, plasma samples for patient MM were pooled and extracted with ethyl acetate. This procedure enabled us to compare plasma concentration of DHT using both methods of detection. The values were 0.609 and 0.598 mg/L using UV- and fluorescence-HPLC, respectively.

The electron-impact GC-MS properties of TBZ and DHT collected from UV-HPLC eluants of plasma samples were identical to those of the respective authentic standards. The major fragments, m/z (% relative abundance), for TBZ included: 317 (M^+, 16), 316(18), 274(40), 261(78), 260(33), 232(18), 192(25), 191(100), 190(32), 176(35); and for DHT included: 319 (M^+, 55), 318(48), 274(53), 232(36), 206(28), 205(100), 192(27), 191(98), 190(24), 176(24). The CI-mass spectra indicated quasi-molecular ions of m/z of 318 and 320 for TBZ and DHT, respectively.

Figure 2-5 depicts the time course of TBZ and DHT in plasma of two patients following oral administration of TBZ. For the unidentified compounds, only the peak area ratio (compound /IS) could be plotted versus time. The time-course of TBZ and DHT following intravenous administration of TBZ to rats is shown in Fig. 2-4.

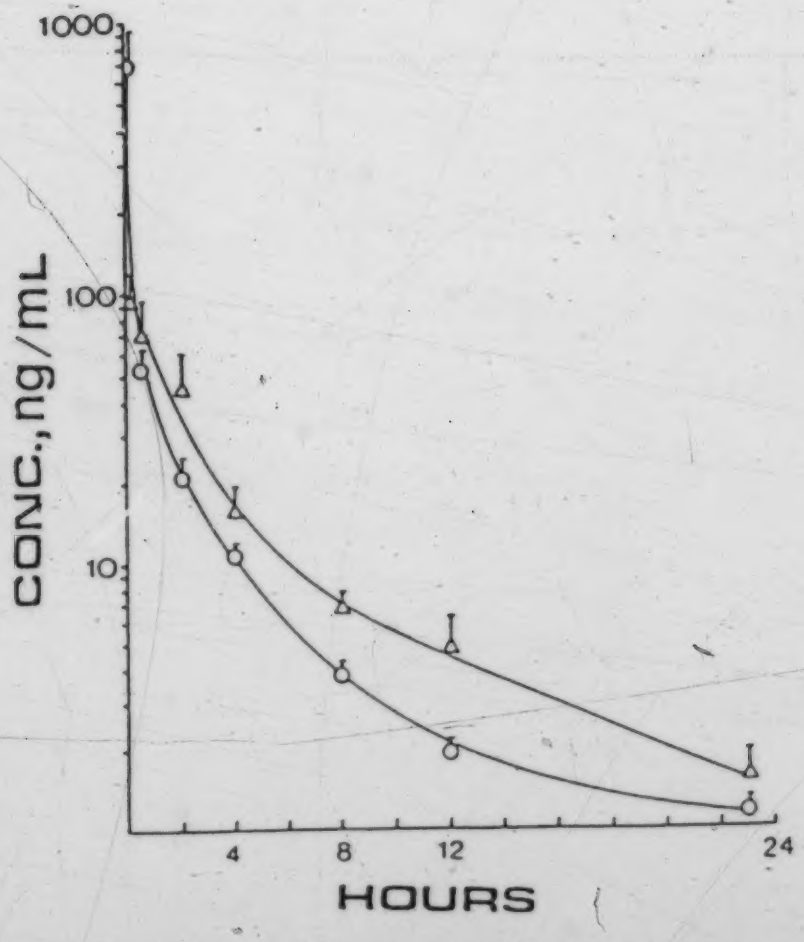


Fig. 2-4. Mean plasma tetrabenazine (O) and dihydrotetrabenazine (Δ) concentration-time curves following iv administration of 1 mg/kg of the drug to four rats. Bars represent standard deviations.

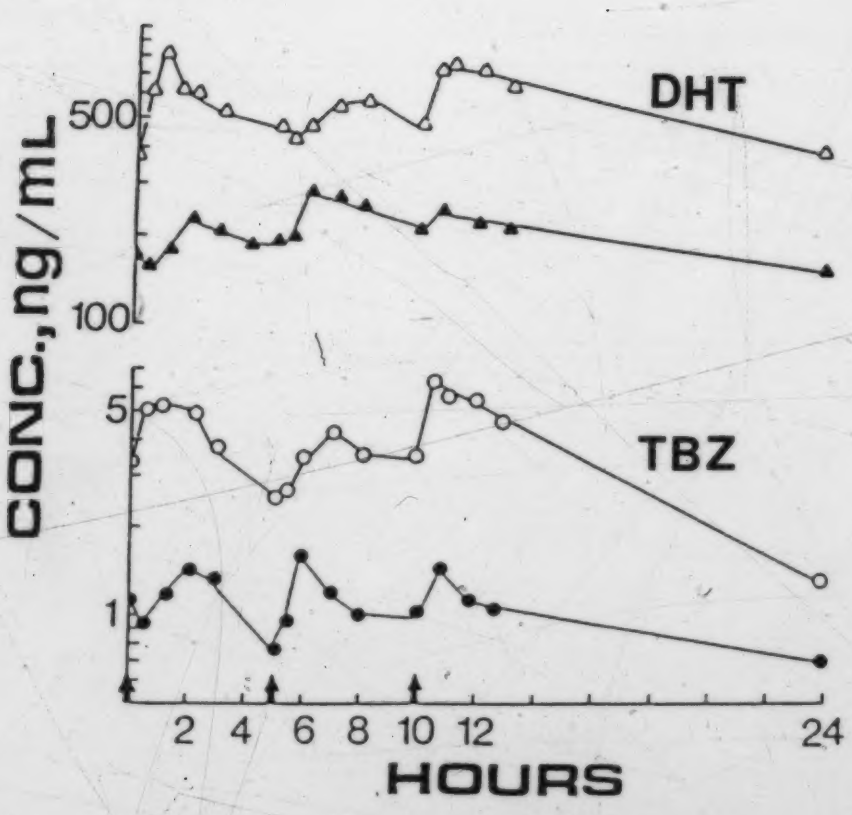


Fig. 2-5. Steady-state time-course of tetrabenazine (TBZ) and its dihydro metabolite (DHT) in patients receiving 25 mg (closed symbols) and 37.5 mg (open symbols) tid, respectively. Arrows indicate times of administration.

This method can be utilized to monitor plasma concentrations and delineate pharmacokinetics of tetrabenazine and its dihydro metabolite in rats and also in patients receiving the recommended dosage of the drug.

2.4. References

1. A. Pletscher, A. Brossi, and K. F. Gey: Benzoquinolizine Derivatives: A New Class of Monoamine Decreasing Drugs with Psychotropic Action. Int. Rev. Neurobiol. **4**, 275-306 (1962)

2. A. Brossi, H. Lindlar, M. Walter, and O. Schnider: Syntheseversuche in der Emetin-Reihe. 2-Oxohydrobenzo[a]chinolizine. Helv. Chim. Acta **41**, 119-139 (1958).

3. M. Swash, A. H. Roberts, H. Zakko, and K. W. G. Heathfield: Treatment of Involuntary Movement Disorders with Tetrabenazine. J. Neurol. Neurosurg. Psychiatry **35**, 186-191 (1972).

4. J. Jankovic: Treatment of Hyperkinetic Movement Disorders With Tetrabenazine: A Double-Blind Crossover Study. Ann. Neurol. **11**, 41-47 (1982).

5. S. Fahn: Long-Term Treatment of Tardive Dyskinesia With Presynaptically Acting Dopamine-Depleting Agents. Adv. Neurol. **37**,267-276 (1983).

6. D. L. McLellan, R. J. Chalmers, and R. H. Johnson: A Double-Blind Trial of Tetrabenazine, Thiopropazate, and Placebo in Patients with Chorea. Lancet **1**, 104-107 (1974).

7. M. S. Roberts, H. M. Watson, S. McLean, and K. S. Millingen: Determination of Therapeutic Plasma Concentrations of Tetrabenazine and an Active Metabolite by High-performance Liquid Chromatography. J. Chromatogr. **226**, 175-182 (1981).

8. F. Jamali, A. S. Russell, B. W. Berry, and C. Lehmann: High-Performance Liquid Chromatographic Analysis of Tiaprofenic Acid and its Metabolites in Plasma and Urine by Direct Injection. J. Chromatogr. **310**, 327-333 (1984).

9. D. E. Schwartz and J. Rieder: Micromethode de Dosage Spectrofluorimetrique des Benzoquinolizines dans les Liquides et Tissus Biologiques. Clin. Chim. Acta **6**, 453-463 (1961).

10. D. E. Schwartz, H. Bruderer, J. Rieder and A. Brossi: Metabolic Studies of Tetrabenzine, a Psychotropic Drug in Animals and Man. Biochem. Pharmacol. **15**, 645-655 (1966).

11. N. J. Leonard, A. S. Hay, R. W. Fulmer, and V. W. Gash: Unsaturated Amines. III. Introduction of α,β-unsaturation by Means of Mercuric Acetate: $\Delta^{1(10)}$-Dehydroquinolizidine. J. Am. Chem. Soc. **77**, 439-444 (1955).

12. A. Sokolowski and K. G. Wahlund: Peak Tailing and Retention Behaviour of Tricyclic Antidepressant Amines and Related Hydrophobic Ammonium compounds in Reversed-Phase Ion-Pair Liquid

Chromatography on Alkyl-bond Phases. J. Chromatogr. 189, 299-316 (1980).

13. S. G. Weber, W. G. Tramposch: Cation Exchange Characteristics of Silica-Based Reversed-Phase Liquid Chromatographic Stationary Phases. Anal. Chem. 55, 1771-1775.

3. PHARMACOKINETICS OF TETRABENAZINE AND ITS MAJOR METABOLITE IN MAN AND RAT. BIOAVAILABILITY AND DOSE-DEPENDENCY STUDIES*

3.1. Introduction

Tetrabenazine (TBZ), a benzoquinolizine derivative, has been reported to have some beneficial effects in the control of movement disorders (1-7). It has been suggested to be the drug of choice in the treatment of Huntington's chorea (3) and TD (6). Similar to reserpine, TBZ depletes catecholamines and serotonin within the brain (8). Besides, TBZ is shown to inhibit both pre- and postsynaptic dopamine receptors (9,10). The side effects of TBZ are reported to be substantially less than reserpine (11).

Although TBZ was introduced as an antipsychotic agent in 1958, reports on its pharmacokinetics and metabolism in man have been scarce. This might be attributed to the difficulties in measuring very low concentrations of the drug following administration of doses normally used in clinical practice (12). The drug undergoes extensive distribution and metabolism following administration of high doses to rabbit, dog, and man (8,13). Very recently pharmacokinetics of the drug and its dihydro metabolite (DHT) were reported in patients treated for involuntary movement disorders (14). We previously reported a sensitive, selective, and specific HPLC method for determination of TBZ and DHT in plasma samples of man and rat (12). This study deals with the disposition kinetics of TBZ and DHT in patients affected by TD and in rats.

---

* A version of this chapter has been published:
R. Mehvar, F. Jamali, M. W. B. Watson, and D. Skelton. Drug Metab. Dispos. **15**, 250-255 (1987).

3.2. Experimental Section

3.2.1. Materials

Powders of TBZ and DHT and TBZ tablets (Nitoman, 25 mg) were gifts from Hoffmann-LaRoche of Canada (Etobicoke, Ont.). Acetonitrile and water were HPLC grade, while all other reagents were analytical grade.

3.2.2. Patient Study

Four patients with controlled TD participated in the study after giving a written informed consent. The doses of the drug and characteristics of the patients are summarized in Table 3-1. Tetrabenazine tablets were administered at 08:00, 13:00, and 18:00 h. Following at least 2 months of therapy with TBZ, blood samples (3 mL) were taken just prior and then 0.5, 1.0, 2.0, 3.0, and 5.0 h after the first and second doses and 0.5, 1.0, 2.0, 3.0, and 14.0, h after the last daily dose. Plasma was separated and kept frozen at -20°C until analyzed.

3.2.3. Animal Experiments

Male Sprague-Dawley rats weighing 250-300 g were used in this study. The animals were kept in the department at least 3 d prior to surgery. A day before the study, under methoxyflurane (Metofane, Pittman-Moore, Ont.)-induced light anesthesia, jugular veins of the animals were catheterized with Silastic tubing. All animals were housed individually in plastic metabolic cages and food was withheld for 12 h prior to the administration of either TBZ or DHT.

Table 3-1. Characteristics of Patients and TBZ dosage regimen

Patient	Sex	Dose	Age	Weight	Concomitant Drugs
		(mg,tid)	(Yr)	(Kg)	
1	M	25.0	42	120	Chlorpromazine, diazepam
2	M	12.5	58	84	Haloperidol, methyprylon, oxazepam
3	F	37.5	62	75	Acetaminophen, doxepin
4	F	37.5	75	60	Acetaminophen, chloral hydrate, nomifensine

Three h after dosing, the animals were permitted to have free access to food.

Both the drug and DHT were dissolved in a 50 mM solution of HCl, and the pH of the solution was adjusted to 4-5 with sodium hydrogen carbonate before iv or oral administration. All animals were treated between 7:00 and 9:00 a.m.

Single oral doses of 0.5, 1.0, 2.5, 5.0, and 10.0 and an iv dose of 1 mg/kg TBZ were each administered to four to six rats. Single iv doses of 1 mg/kg DHT were administered to four rats.

Blood samples (0.25 mL) were collected through the jugular vein cannula at 0, 10, and 30 min and at 1.5, 3, 5, 8, 12, and 24 h following administration of TBZ or DHT. The serum was separated and frozen at -20^{o}C until analyzed. Total urine output was collected up to 24 h. These experiments were carried out following a preliminary study on two isolated rat livers which were perfused with Krebs bicarbonate buffer (pH 7.4) containing 60 ng/mL TBZ at a flow rate of 2 mL/min for each g of liver. Two male Sprague-Dawley rats weighing 300 and 315 g were used as liver donors. The average of two consecutive determinations at 5 and 10 min after commencement of the perfusion was calculated as the input concentration. The output concentration was determined by averaging four steady-state determinations at 10, 20, 30, and 40 min post-perfusion. No red blood cells or protein were added to the perfusate.

3.2.4. Blood:Plasma Concentration Ratio

Drug-free heparinized blood was obtained from Sprague-Dawley rats and normal volunteers. Tetrabenazine (dissolved in isotonic

phosphate buffer pH 7.4) was added to the blood to make final concentrations of 10 and 25 ng/mL in the human blood and 125 ng/mL in the rat blood (n = 4). The samples were incubated at 37°C for 30 min and then were centrifuged utilizing a Fisher model 235A microcentrifuge (Edmonton, AB) for 5 min. The resultant plasma was assayed for TBZ. The added blood:measured plasma concentration was considered to be the blood:plasma TBZ concentration ratio.

3.2.5. Assay Procedure

The concentrations of TBZ and DHT in plasma and urine were determined using an HPLC method developed in our laboratory (12) after some modifications. To 0.1-0.4 mL of plasma or urine were added 0.1 mL of a 0.2 M solution of sodium hydroxide and 0.05 mL of benzoquinoline solution (50 ng/mL) as the internal standard. The drug, its metabolites, and the internal standard were extracted from the urine and plasma by adding 0.8 mL of ethyl acetate and vortexing the tubes for 30 sec. After centrifugation, the supernatant was evaporated in a Savant Speed Vac concentrator (Emerston Instrument, Scarborough, Canada). The drug and its metabolite were then dehydrogenated to make them sensitive to fluorescence detection. To do this, 0.2 mL of a mercuric acetate solution (1 g mercuric acetate, 5 mL acetic acid, 5 g sodium acetate trihydrate, 45 mL water and 50 mL methanol) was added to the residue and the tubes were heated for 1 h at 110°C. The fluorescence detector (GM 970, Kratos, NJ) was set at excitation and emission wavelengths of 265 and 418 nm, respectively. The chromatography was carried out on a Partisil 5 ODS-3 (Whatman, N.J.) using a mobile phase consisting of water,

acetonitrile, acetic acid, and triethylamine (66:32:2:0.2) and flow rate of 0.6 mL/min at 60°C.

3.2.6. Pharmacokinetic Analysis

Model-independent methods (15) were used to estimate the clearances of the iv (Cl_s) and oral (Cl_o) doses. The volume of distribution (V_d) was determined by the area method (15). The AUC was calculated using the linear trapezoidal rule with end correction, where applicable. In the patients study the AUCs were calculated for a 24-h period and the Cl_o was calculated by dividing the total daily dose by the corresponding AUC. The extraction ratio (e) and systemic bioavailability (f) in patients were estimated using the following equations, assuming complete absorption, hepatic blood flow of 1.28 $L.h^{-1}.kg^{-1}$ (15), complete elimination through liver metabolism (16), and blood:plasma concentration ratios of 1 (17):

$$Cl_o = Dose/AUC_o = Cl_s/1-e = Q.e/1-e$$

$$f = 1-e$$

where AUC_o and Q are the AUC after oral administration and liver blood flow, respectively. In rats f was obtained by the following equation:

$$f = AUC_o/AUC_{iv}$$

where, AUC_o and AUC_{iv} are the mean AUCs after po and iv administration of the same dose of TBZ to rats. Assuming complete absorption, the following equation was utilized to estimate Q in rats (16, 18):

$$Q = (Cl_s.Cl_o)/(Cl_o - Cl_s)$$

3.3. Results

3.3.1. Patients Study

A representative semilogarithmic plot of plasma concentrations of TBZ and DHT in patient 4 is shown in Figure 3-1, and the kinetic indices of all of the patients are summarized in Table 3-2.

The observed AUCs of DHT were substantially larger than those of the parent drug and the F values in these patients indicate an extensive presystemic metabolism of TBZ. Only trace amounts of TBZ were found in the patients urines.

3.3.2. Animal Experiments

Figure 3-2 depicts chromatograms of the inlet and outlet solutions for one of the isolated rat liver perfusions. The outlet:inlet ratios of average TBZ concentrations were 0.05 and 0.06 in the two cases. In addition to TBZ and DHT, the effluent contained at least 3 other peaks, presumably representing other metabolites of TBZ.

The time courses of TBZ and DHT after iv and po administration of single 1 mg/kg doses of TBZ to rats are shown in Figure 3-3. In all animals the first plasma sample, taken 10 min after oral doses, contained the peak concentrations (C_{max}). For DHT, however, C_{max} was attained in 30 min. The initial decrease in the plasma concentration of TBZ was very rapid following both routes of administration, indicating multicompartment disposition kinetics.

The plots of the means of TBZ and DHT AUCs versus corresponding oral doses of 0.5-10 mg/kg of TBZ are depicted in Figure 3-4. The best-fit lines for the observed data were AUC(TBZ) = 7.42 + 31.4

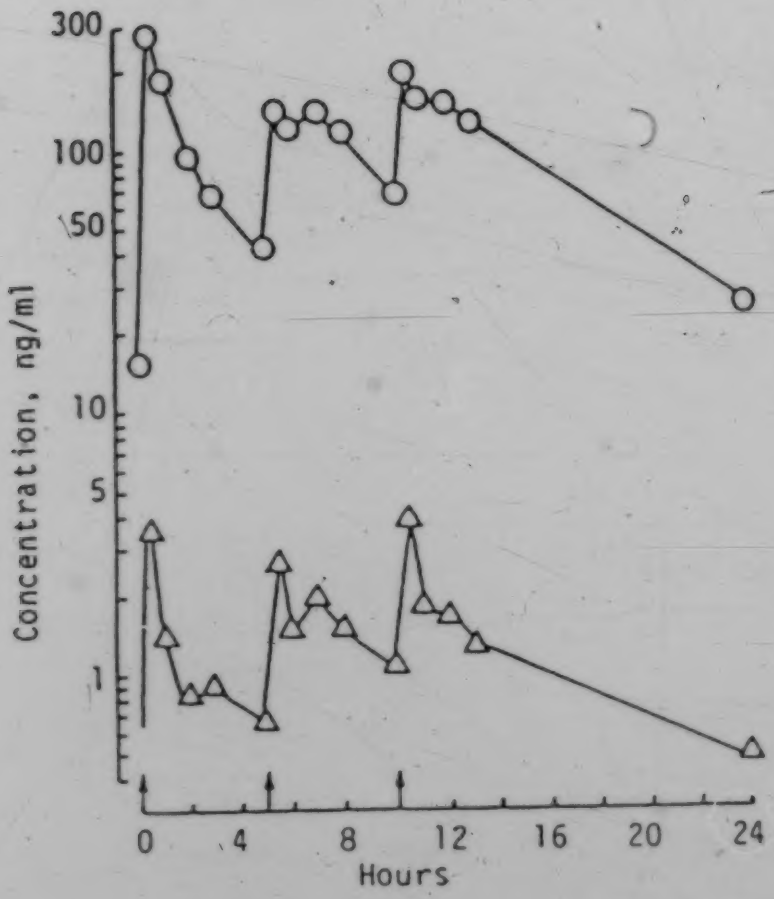


Fig. 3-1. Plasma TBZ (Δ) and DHT (O) concentrations versus time in patient 4. The arrows indicate the time of dosing.

Table 3-2. Pharmacokinetic indices of TBZ and DHT in patients under treatment with TBZ

Patient	AUC_{0-24}		AUC_{DHT}/AUC_{TBZ}	Cl_o	f	DHT in Urine (0-24 h,%dose)
	TBZ	DHT				
	(ng/mL)h			(mL/min)/kg		
1	24.2	4810	199	431	0.05	8.47
2	29.5	4550	154	252	0.08	2.71
3	84.8	13400	158	299	0.07	-
4	28.6	2360	82.6	1090	0.02	3.23

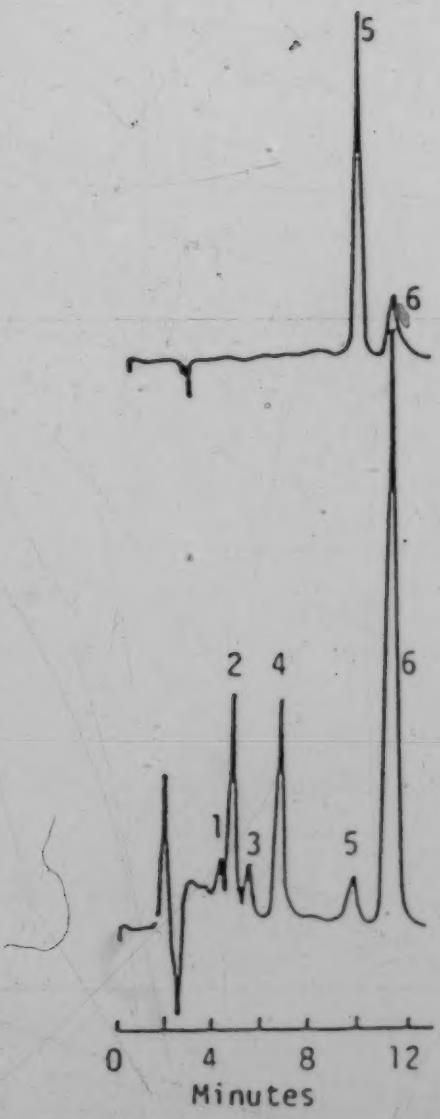


Fig. 3-2. HPLC of a 60 ng/mL solution of TBZ before (top) and after (bottom) one pass through an isolated rat liver. Peaks: 1, 2 and 3, unkown metabolits; 4, DHT; 5, TBZ; 6, internal standard. Different volumes of solutions are injected into the instrument.

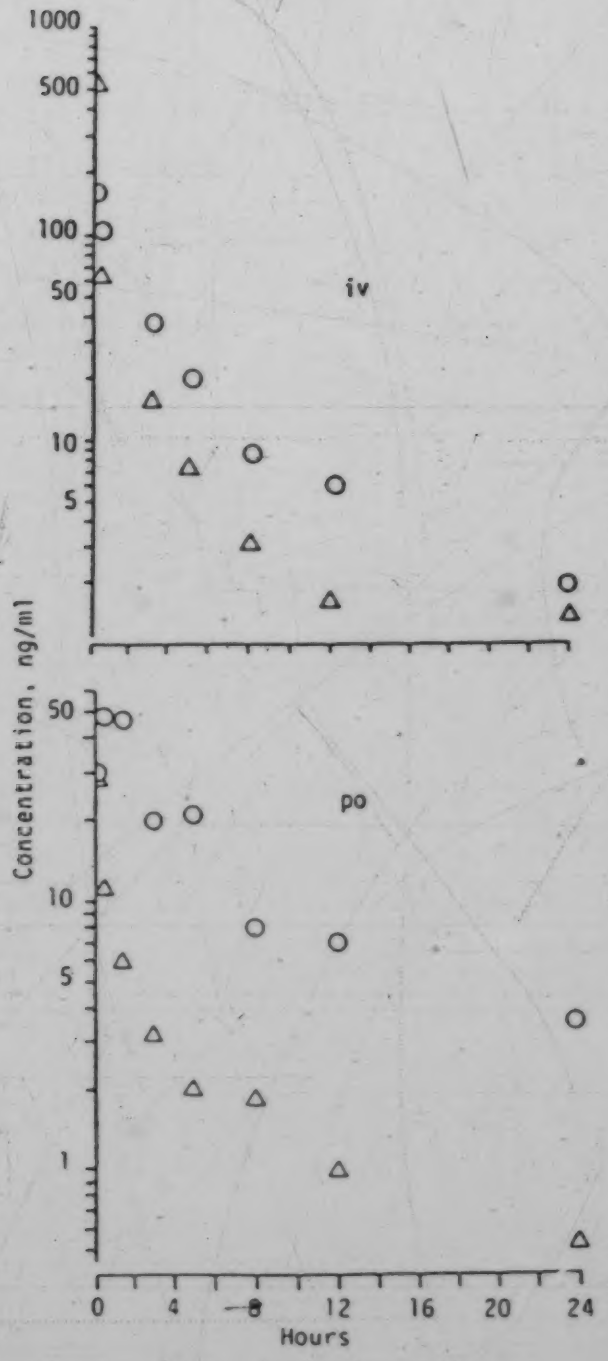


Fig. 3-3. Individual plasma TBZ (△) and DHT (○) concentration-time curves following iv and po administration of 1 mg/kg doses of TBZ to rats.

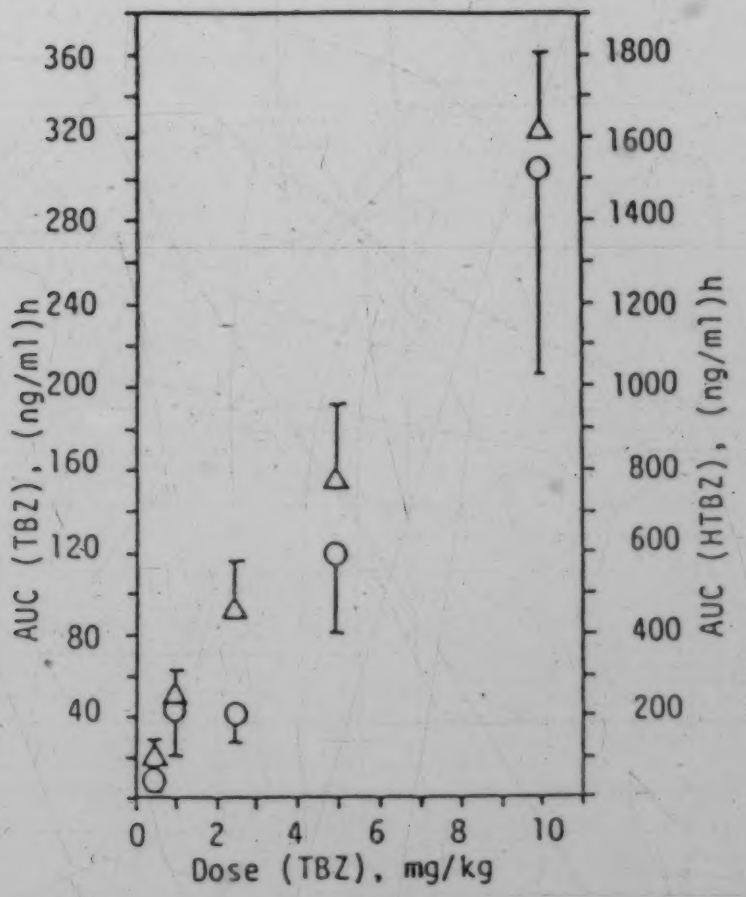


Fig. 3-4. The observed mean AUC of TBZ (△) and DHT (○) versus the given doses of TBZ in rats. Bars represent standard deviations.

dose(TBZ) and AUC(DHT) = -44.4 + 150.7 dose(TBZ), with correlation coefficients of 0.998 for TBZ and 0.986 for DHT. The intercepts of these lines were not significantly different from zero. The population Cl_0 estimated for TBZ from the inverse of the slope of the Figure 3-4 was 530 $mL.min^{-1}.kg^{-1}$.

The mean clearance values of TBZ and the ratios of the AUC of DHT over that of TBZ for each dose are presented in Table 3-3. The mean clearance value obtained after iv administration of TBZ is severalfold smaller than that after po administration. Following an iv dose, AUC values for TBZ and DHT were 285 ± 30.5 and 253 ± 64.1 (Mean ± SD) $ng.mL^{-1}.h$, respectively. Following oral administration of 1 mg/kg of TBZ to the rat, f was 0.17.

Figure 3-5 depicts the mean plasma concentration-time profile of DHT after administration of single iv doses of 1 mg/kg DHT to the rat. The pharmacokinetic indices of DHT in the rat are presented in Table 3-4. Similar to TBZ, the observed Cl_s of DHT was very high and approached the liver blood flow. In the rat, DHT was metabolized extensively, as only approximately 2% of the dose was excreted unchanged in urine during a 24-h collection period (Table 3-4).

3.3.3. Blood:Plasma TBZ Concentration Ratio

In the rat blood a ratio of 0.983 ± 0.033 (Mean ± SD) was observed for concentration of 125 ng/mL. In the human blood the ratios were 1.13 ± 0.107 and 1.19 ± 0.042 for concentrations of 10 and 25 ng/mL, respectively. Therefore, in our calculations we assumed a ratio of 1 for TBZ.

Table 3-3. Metabolite:drug AUC ratios and the clearance of TBZ following different doses of TBZ in the rat

Dose	n	AUC_{DHT}/AUC_{TBZ}	Cl
(mg/kg)			(mL/min)/kg
0.50 (po)	5	2.4 (1.1)[a]	426 (100)
1.0 (po)	6	4.7 (4.3)	348 (75)
2.5 (po)	4	2.3 (1.1)	475 (120)
5.0 (po)	4	3.8 (0.74)	567 (160)
10 (po)	4	6.3 (5.3)	623 (350)
1.0 (iv)	6	0.97 (0.3)	58.9 (6.0)

[a] Numbers in parentheses are standard deviations.

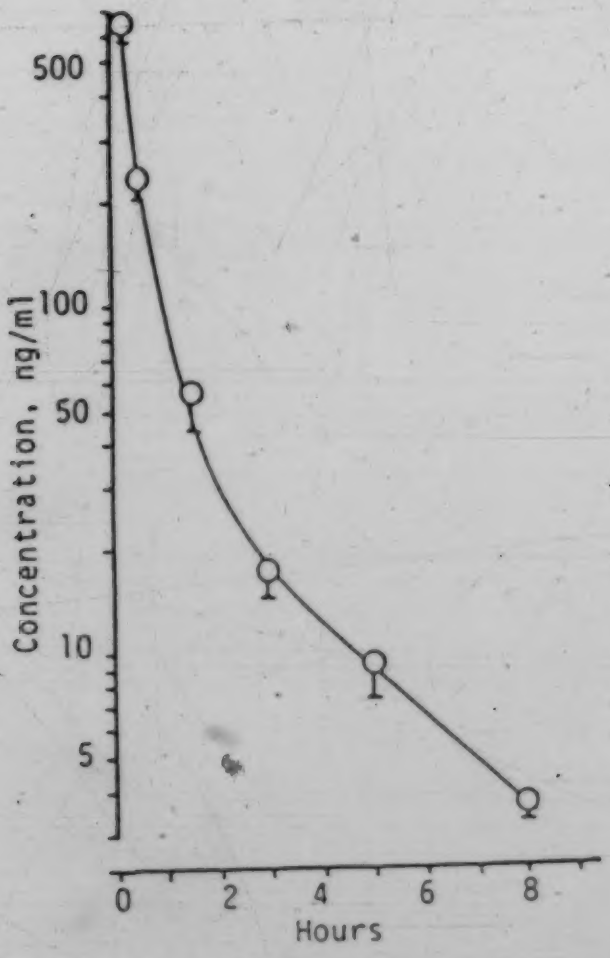


Fig. 3-5. Mean plasma DHT concentration-time course following iv administration of 1 mg/kg of DHT to rats. Bars represent standard deviations.

Table 3-4. Pharmacokinetic indices of DHT following iv administration of 1 mg/kg DHT to four rats

Rat	$AUC_{0-\infty}$	Cl_s	$t_{1/2}$	V_d	%Recovered in Urine[a]
	(ng/mL)h	(mL/min)/kg	(h)	(l/kg)	
1	508	32.8	1.6	4.6	1.9
2	389	42.8	1.5	5.7	1.3
3	293	56.8	2.8	14	3.6
4	377	44.2	1.8	7.0	1.5
X	392	44.2	1.9	7.8	2.1
SD	89	9.8	0.6	4	

[a] 0-24 h.

3.4. Discussion

Oral administration of 12.5 to 37.5 mg TBZ tid to patients resulted in very low plasma concentrations of the parent drug (0.240-6.22 ng/mL) and relatively high concentrations of DHT (15.9-801 ng/mL). Due to the unavailability of authentic samples, the concentrations of other metabolites could not be measured, but their high fluorescence intensities (12) may be indicative of their high plasma concentrations. No unchanged TBZ was found in the urine of the patients, indicating extensive metabolism of the drug. Following iv administration of doses of 50 mg/kg TBZ to rabbits, Quinn et al. (8) reported excretion of less than 2% of unchanged drug in the urine. In another study 6 urinary metabolites of TBZ were identified in rabbit, dog and man (13). Apparently in all the species the elimination of TBZ takes place only through metabolism. Furthermore, the observed Cl_0 of 252-1090 $mL.min^{-1}.kg^{-1}$ (Table 3-2) are considerably higher than the average liver blood flow of 1500 $mL.min^{-1}$ in a 70 kg man (15). This may indicate that the extensive metabolism of TBZ takes place during the first pass through the liver and/or the gut.

Conventionally, systemic availability is determined by the ratio of the AUCs following extra- and intravascular drug administration (15). To us the administration of iv TBZ, however, did not seem to be ethical as it could result in dangerously high concentrations of the intact drug. Alternatively, the f values in the patients were estimated from the oral data. The calculated f was 0.06 $\pm$ 0.03 (Mean $\pm$ SD) which was very close to 0.049 $\pm$ 0.032 reported recently (14) using data generated following iv and oral doses. The

possibility of the presystemic biotransformation of TBZ was then examined in the rat.

The preliminary study with the isolated rat livers indicates a very extensive metabolism of TBZ after the first passage through the liver (Figure 3-2).

Following iv administration of 1 mg/kg TBZ to rats, the mean Cl_s of TBZ is 58.9 ± 6.01 $mL.min^{-1}.kg^{-1}$ (Table 3-3) which is very close to the reported average rat liver blood flow of 58.8 $mL.min^{-1}.kg^{-1}$ (19). Our estimated Q was 67.4 ± 2.32 $mL.min^{-1}.kg^{-1}$. The difference between our calculated Q and that reported in the literature is too small to suggest a significant extrahepatic elimination. These findings indicate a very high liver extraction ratio and a perfusion-limited clearance (20). To rule out the possibility of poor absorption, the ratio of the AUC values of DHT obtained following oral and iv doses of TBZ was estimated. Assuming exclusive clearance of TBZ by the liver, a value of 0.84 was observed, indicating an almost complete absorption (21). The very low bioavailability of TBZ after oral administration of the drug to the rat, therefore, appears to be due to a presystemic metabolism in the liver rather than an incomplete absorption.

Large interindividual variations in the availability and clearance values have been reported for drugs with a perfusion-limited clearance such as lidocaine (22,23), verapamil (24), and labetalol (25). Similarly the pharmacokinetic indices of TBZ and DHT in both man and the rat also showed large variations (Tables 3-2 and 3-3).

For rapidly absorbed drugs such as TBZ, relatively high concentrations of the drug appear in the portal vein following oral administration (17). This may result in a saturable first-pass metabolism of the drug if the responsible hepatic enzymes are capacity limited. Consequently, the oral availability of propranolol (26), quinidine (27), and verapamil (28) are reported to increase upon increasing the dose or following repeated dosing. An investigation of the dose dependency of the pharmacokinetics of TBZ after oral doses, therefore, seemed necessary. In the rat, higher doses of TBZ resulted in proportionally greater AUCs for both the drug and its major metabolite, DHT (Figure 3-4). We realize that this may not hold true in man. However, within the dosage range of 12.5 to 37.5 mg tid, the estimated f values in the patients were substantially low (Table 3-2). Thus, it does not seem likely that the saturation of the first-pass metabolism of TBZ occurs in this dosage range.

The major discrepancy between the pharmacokinetic pattern of TBZ in the patients and rats was the substantially greater AUC_{DHT}/AUC_{TBZ} in the patients (Tables 3-2 and 3-3). This may result from a more rapid elimination and/or less extensive formation of the metabolite in the rat as compared to man. Administration of DHT to rats was, therefore, carried out to investigate the pharmacokinetics of DHT in the rat. The plasma profile of DHT after iv doses follows characteristics of a multiexponential pharmacokinetic model (Fig. 3-5) with a very short terminal half-life (Mean = 1.9 h) (Table 3-4). The Cl_s of DHT, although less than TBZ, is relatively high and approaches the rat liver blood flow

(19). Consequently one might expect a very high extraction ratio for DHT and a low availability after oral administration to rats (16). Hence, a sequential first-pass elimination for DHT after oral doses of TBZ, similar to what was proposed for phenacetin by Pang and Gillette (29), might be expected. Roberts et al. (14) reported a mean value of 0.81 for the ratio of AUCs of DHT following oral and iv administration of TBZ. They erroneously interpreted this as being the systemic availability of the metabolite, and attributed their finding to a low hepatic extraction for DHT. The above ratio does not provide information on the availability of the metabolite, as it may be only an indication of the extent of the absorption of the parent drug (21,30). These authors (14) also reported a value of 35 ± 35 (Mean ± SD) as their observed AUC_{DHT}/AUC_{TBZ} in the patients following po administration of TBZ. This value is substantially less than our value of 148 ± 48.5 (Table 3-2). The discrepancy might be due to the fact that they, contrary to their explanation, averaged all the values obtained following both single and multiple po doses of TBZ (Table 4, ref. 14).

The fraction of a dose which is converted to a specific metabolite, F_m, might be obtained by the following equation (15):

$$F_m = AUC(m,D)/AUC(m)$$

where AUC(m,D) and AUC(m) are the AUC of the metabolite formed after iv administration of its precursor and that after administration of an equimolar dose of the preformed metabolite, respectively. The metabolite had an F_m value of 0.69. This may, however, be an underestimation of F_m, as the above equation may not hold valid for metabolites such as DHT which undergo extensive presystemic

metabolism (29-31). In such cases a more accurate approach is to compare the AUC(m,D) with the AUC(m)o, where the latter is the AUC of the metabolite following its oral administration. Regardless of the elimination route of the metabolite, however, it is assumed that it is formed in the liver (30). Nevertheless, in spite of relatively low plasma concentrations of DHT in rats, a major fraction (at least 69%) of TBZ seems to be converted to DHT. The difference in the ratios of the AUC of DHT to TBZ between the rat and man, therefore, is likely due to a higher clearance of DHT in the rat rather than a small F_m.

Considering the relatively high concentrations of DHT, and perhaps other metabolites of TBZ in humans, one may pose the question of whether "the efficacy of TBZ administration is due to the very low concentrations of the parent drug and/or the relatively high concentrations of the metabolites." This is especially important because in vitro studies have shown pharmacological activities similar to those of TBZ for DHT (32). It must be mentioned, however, that as the site of action of the drug is the central nervous system, for DHT to be effective, it must pass through the blood-brain barrier. This issue is now being investigated in our laboratory.

For highly extracted drugs such as TBZ, one might expect substantial changes in the oral availability and pharmacokinetics in patients with altered liver function and liver blood flow (33). In clinical terms, this issue is worthy of consideration, as the patients affected by TD are mainly from the elderly population (34) who are more prone to conditions such as liver dysfunction.

3.5. References

1. E. Brandrup: Tetrabenazine Treatment in Persistent Dyskinesia Caused by Psychopharmaca. Am. J. Psychiatry **118**, 551-552 (1961).

2. H. Kazamatsuri, C. P. Chien, and J. O. Cole: Long-Term Treatment of Tardive Dyskinesia With Haloperidol and Tetrabenazine. Am. J. Psychiatry **130**, 479-483 (1973).

3. D. L. McLellan, R. J. Chalmers, and R. H. Johnson: A Double-Blind Trial of Tetrabenazine, Thiopropazate, and Placebo in Patients With Chorea. Lancet **1**, 104-107 (1974).

4. D. Kingston: Tetrabenazine for Involuntary Movement Disorders. Med. J. Aust. **1**, 628-630 (1979).

5. J. Jankovic: Treatment of Hyperkinetic Movement Disorders With Tetrabenazine: A Double-Blind Crossover Study. Ann. Neurol. **11**, 41-47 (1982).

6. S. Fahn: Long-Term Treatment of Tardive Dyskinesia With Presynaptically Acting Dopamine-Depleting Agents. Adv. Neurol. **7**, 267-276 (1983).

7. M. Bartels and E. Zoller: Tetrabenazine (Nitoman) Therapy of Chronic Spontaneous Oral Dyskinesia. Eur. Arch. Psychiatry Neurol. Sci. **234**, 172-174 (1984).

8. G. P. Quinn, P. A. Shore, and B. B. Brodie: Biochemical and Pharmacological Studies of RO 1-9569 (Tetrabenazine), a Non-Indole Tranquilizing Agent With Reserpine-Like Effects. J. Pharmacol. Exp. Ther. **127**, 103-109 (1959).

9. R. Kuczenski: Differential Effects of Reserpine and Tetrabenazine on Rat Striatal Synaptosomal Dopamine Biosynthesis and Synaptosomal Dopamine Pools. J. Pharmacol. Exp. Ther. **20**, 357-367 (1977).

10. A. Reches, R. E. Burke, C. M. Kuhn, M. N. Hassan, V.R. Jackson, and S. Fahn: Tetrabenazine, an Amine Depleting Drug, Also Blocks Dopamine Receptors in Rat Brain. J. Pharmacol. Exp. Ther. **225**, 515-521 (1983).

11. B. O. Mikkelsen: Tolerance of Tetrabenazine During Long-Term Treatment. Acta. Neurol. Scand. **68**, 57-60 (1983).

12. R. Mehvar, F. Jamali, M. W. B. Watson, and D. Skelton: Direct High-Performance Liquid Chromatography of Tetrabenazine and Its Metabolite in Plasma of Humans and Rats. J. Pharm. Sci. **75**, 1006-1009 (1986).

13. D. E. Schwartz, H. Bruderer, J. Rieder, and A. Brossi: Metabolic Studies of Tetrabenazine, a Psychotropic Drug in Animals and Man. Biochem. Pharmacol. **15**, 645-655 (1966).

14. M. S. Roberts, S. McLean, K. S. Millingen, and H. M. Galloway: The Pharmacokinetics of Tetrabenazine and Its Hydroxy Metabolite in Patients Treated for Involuntary Movement Disorders. Eur. J. Clin. Pharmacol. **29**, 703-708 (1986).

15. M. Gibaldi and D. Perrier: "Pharmacokinetics" Marcell Dekker, New York, 1982.

16. G. R. Wilkinson and D. G. Shand: A Physiological Approach to Hepatic Drug Clearance. Clin. Pharmacol. Ther. **18**, 337-390 (1975).

17. S. M. Pond and T. N. Tozer: First-Pass Elimination, Basic Concepts and Clinical Consequences. Clin. Pharmacokinet. 9, 1-25 (1984).

18. C. S. Reilly, J. Biollaz, R. P. Koshakji, and A. J. J. Wood: Enprostil, in Contrast to Cimetidine, Does Not Inhibit Propranolol Metabolism. Clin. Pharmacol. Ther. **40**, 37-41 (1986).

19. H. Harashima, Y. Sawada, Y. Sugiyama, T. Iga, and M. Hanano: Analysis of Nonlinear Tissue Distribution of Quinidine in Rats by Physiologically Based Pharmacokinetics. J. Pharmacokinet. Biopharm. **13**, 425-440 (1985).

20. C. F. George: Drug Kinetics and Hepatic Blood Flow. Clin. Pharmacokinet. **4**, 443-448 (1979).

21. K. S. Pang: A Modern View of Metabolite Kinetics. In "Pharmacokinetics and Pharmacodynamics of Psychoactive Drugs" (G. Barnett and C. N. Chiang, eds.), pp. 323-379. Biomedical Publications, Foster City, CA 1985.

22. P. N. Bennette, L. J. Aarons, M. R. Bending, J. A. Steiner, and M. Rowland: Pharmacokinetics of Lidocaine and Its Deethylated Metabolite: Dose and Time Dependency Studies in Man. J. Pharmacokinet. Biopharm. **10**, 265-281 (1982).

23. W. A. Ritchel, H. Elconin, G. J. Alcorn, and D. D. Denson: First-Pass Elimination of Lidocaine in the Rabbit After Peroral and Rectal Route of Administration. Biopharm. Drug Dispos. 6, 281-290 (1985).

24. M. Schomerus, B. Spiegelhalder, B. Stiern, and M. E. Eichelbaum: Physiological Disposition of Verapamil in Man. Cardiovasc. Res. 10, 605-612 (1976).

25. J. J. McNeil, A. E. Anderson, and W. J. Louis: Pharmacokinetic and Pharmacodynamic Studies of Labetalol in Hypertensive Subjects. Br. J. Clin. Pharmacol. **8**, 157s-161s (1979).

26. J. W. McBride, H. G. McCoy, M. L. Rylander: Variable First-Pass Elimination of Propranolol Following Single and Multiple Oral Doses in Hypertensive Patients. Eur. J. Drug Metab. Pharmacokinet. **7**, 183-189 (1982).

27. M. G. Horning, and K. S. Pang: Dose-Dependent Kinetic of Quinidine in the Perfused Rat Liver Preparation, Kinetics of Formation of Active Metabolite. Drug Metab. Dispos. **10**, 568-572 (1982).

28. J. G. Wagner, G. J. Szpunar, and J. J. Ferry: A Nonlinear Physiologic Pharmacokinetic Model: I.Steady-State. J. Pharmacokinet. Biopharm. **13**, 73-92 (1985).

29. K. S. Pang and J. R. Gillette: Sequential First-Pass Elimination of a Metabolite Derived From a Precursor. J. Pharmacokinet. Biopharm. **7**, 275-290 (1979).

30. K. S. Pang: Metabolite Pharmacokinetics: The Area Under the Curve of Metabolite and the Fractional Rate of Metabolism of a Drug After Different Routes of Administration for Renally and Hepatically Cleared Drugs and Metabolites. J. Pharmacokinet. Biopharm. **9**, 477-487 (1981).

31. K. S. Pang and J. R. Gillette: Theoretical Relationships Between Area Under the Curve and Route of Administration of Drugs and Their Precursors for Evaluating Sites and Pathways of Metabolism. J. Pharm. Sci. **67**, 703-704 (1978).

32. D. Scherman, P. Jaudon, and J. P. Henry: Characterization of Monoamine Carrier of Chromaffin Granule Membrane by Binding of [2-^{3}H]dihydrotetrabenazine. Proc. Nat. Acad. Sci. U.S.A. 80, 584-588 (1983).

33. A. J. J. Wood, D. M. Kornhauser, G. R. Wilkinson, D. G. Shand, and R. A. Branch: The Influence of Cirrhosis on Steady-State Blood Concentrations of Unbound Propranolol After Oral Administration. Clin. Pharmacokinet. **3**, 478-487 (1978).

34. S. Mukherjee, A. M. Rosen, C. Cardenas, V. Varia, and S. Olarte: Tardive Dyskinesia in Psychiatric Outpatients. Arch. Gen. Psychiatry **39**, 466-469 (1982).

4. CONCENTRATION-EFFECT RELATIONSHIPS OF TETRABENAZINE AND DIHYDROTETRABENAZINE IN THE RAT*

4.1. Introduction

The effectiveness of tetrabenazine (TBZ) in the control of hyperkinetic disorders such as tardive dyskinesia (TD) and Huntington's chorea (HC) is now well established (1-6). The clinical effects of TBZ have been attributed to its ability to deplete brain catecholamines and serotonin (5-HT)(7), as well as blockade of both pre- and postsynaptic dopamine (DA) receptors (8-10). Limited available pharmacokinetic data indicate that TBZ undergoes a very extensive metabolism resulting in formation of 6 metabolites (11), and has a low oral bioavailability of 6% in humans (12,13) and 17% in rats (12). Following po administration of therapeutic doses of TBZ to patients with TD, the steady-state area under the plasma concentration-time curve of tetrabenazine's major metabolite, dihydrotetrabenazine (DHT), was shown to be 82.6 - 199 fold higher than that of the parent drug (12). In vitro studies indicate that DHT, like TBZ, is capable of depleting catecholamines from their storage sites (14,15). However, the extent of its in vivo effects has been questioned as, utilizing chromaffin granule membranes, the activity of DHT was shown to be only half of that of TBZ (14). We have previously posed the question of whether the efficacy of administration of TBZ is due to the very low concentrations of TBZ and/or the relatively high concentrations of DHT (12). Roberts

---

* A version of this chapter has been published: R. Mehvar and T. Jusko, J. Pharm. Sci., 76, 461-465 (1987).

et al. (13) have also suggested the possibility of DHT being "more therapeutically important than" TBZ. In this study, attempts were made to correlate the serum concentrations of TBZ and DHT with their corresponding brain concentrations following ip injections of TBZ and DHT, and to compare the pharmacodynamics of TBZ and DHT in the rat.

4.2. Experimental Section

4.2.1. Materials

Dopamine (DA), norepinephrine (NE), 5-HT, and 3,4-dihydroxybenzylamine were purchased as either free or salt forms from Sigma (St. Louis, MO). Tetrabenazine (lot # 61-84) was a gift from Hoffmann-LaRoche of Canada (Etobioke, Ont.), while DHT was prepared by reduction of TBZ by sodium borohydride in methanol (15). The melting point and mass spectrum of the synthesized powder were identical to those of an authentic sample supplied by Hoffmann-LaRoche (12). 5,6-Benzoquinoline (B.D.H. laboratory, England) was used as internal standard (IS) in the assay of TBZ and DHT in the serum or brain samples. All the other reagents were analytical grade, except acetonitrile which was HPLC grade.

4.2.2. Doses and Sample Collection

Male, Sprague-Dawley rats weighing 200-250 g were kept in the department at least 3 d before dosing. At 0.5, 2, 5, and 12 h following ip injections of 3 mg/kg of either TBZ or DHT, rats (n = 4) were decapitated. The brains were rapidly removed and dissected into two identical halves, transferred to tubes kept on dry ice and

Serum samples were obtained from blood by centrifugation and stored at -20°C until analysis for TBZ and DHT.

4.2.3 Assay Procedure

Serum concentrations of TBZ and DHT were analyzed utilizing a previously reported HPLC method (12,16). The brain concentrations of TBZ and DHT were measured by a method which is described here. One half of each brain sample was homogenized in glass tubes with a motorized tissue homogenizer (Bodine Electric, Chicago, IL, USA) after addition of 4 mL of an ice cold 0.5 M solution of perchloric acid containing 0.5% mercuric acetate as a protein precipitant and 0.2 mL of IS (50 ng/mL). One mililiter of the homogenate was transferred to a microcentrifuge tube and centrifuged (microcentrifuge model 235A, Fisher, Edmonton, AB, Canada) for 10 min. The supernatant was then transferred to a glass tube and excess sodium carbonate was added. The drug and its metabolite were then extracted with 3 mL of ethyl acetate. The organic layer was evaporated to dryness in a Savant Speed Vac Concentrator/Evaporator (Emerston Instrument, Scarborough, Canada). The dehydrogenation of TBZ and DHT and chromatography conditions were the same as described previously (12,16).

The concentrations of TBZ and DHT were calculated from standard curves obtained by spiking drug-free rat brains with known amounts of TBZ and DHT to final concentrations of 5 to 500 ng/g for TBZ, and 10 to 1000 ng/g for DHT. Excellent linearity (r = 0.990-0.999) was observed between ratios (TBZ/IS and DHT/IS) and their corresponding

<10%. The recovery from brain (n = 4) of TBZ and DHT was estimated by measuring the response in the presence and absence of brain and was found to be 71.6 ± 1.7% and 68.3 ± 1.9% for TBZ and 64.6 ± 2.1% and 68.2 ± 2.7% for DHT at concentrations of 50 and 250 ng/g, respectively.

Concentrations of DA, NE, and 5-HT were determined in the other half of the brain samples using a direct injection HPLC method (17) with a modified mobile phase (55 mM sodium dihydrogen phosphate, 0.85 mM sodium octyl sulfate, 0.37 mM EDTA and 9% acetonitrile, adjusted to pH 3.0 with phosphoric acid). Brain samples were homogenized in 0.1 M solution of perchloric acid containing 0.01% EDTA and 50 ng/mL of 3,4-dihydroxybenzylamine as the IS. The concentrations of amines were determined from a standard curve. Average brain concentrations of the monoamines in eight untreated rats served as baseline values.

4.2.4. Data Analysis

The pharmacologic effects of TBZ and DHT were determined by their ability to decrease brain concentrations of DA, NE, and 5-HT. Brain concentrations of the monoamines were plotted against the corresponding log serum concentrations of TBZ and DHT.

Utilizing the baseline values in the control brains, the percent changes in the concentrations of the amines (E) following treatment with TBZ or DHT was calculated by the following equation:

$$E = [1-(N/N_b)] \times 100$$

where N is the brain concentration of the amine and N_b is corresponding average baseline value of the amine. Plots of C/E

constructed utilizing the following equation, based on the E_{max} model (18):

$$C/E = C/E_{max} + K_d/E_{max}$$

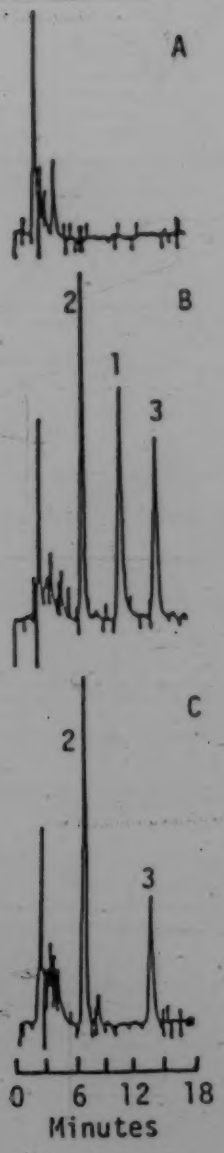
where K_d is the equilibrium dissociation constant which equals the concentration of TBZ or DHT which produces half of the maximum effect (E_{max}). The E_{max} value in each case was determined from the inverse of the slope, and K_d values were calculated by multiplying the y-intercepts by E_{max}.

Utilizing plots of E versus time, the predicted maximum effect after ip administration of 3 mg/kg of TBZ or DHT was calculated (19). The slopes of these lines were also calculated and compared. The data points obtained 12 h after injection were not incorporated in the calculation, as in all cases, the monoamine concentrations returned to the baseline values before this time.

Linear regression was used to examine the relationships between serum and brain concentrations of TBZ or DHT. Homogeneity of the slopes of the plots of E versus time was tested by analysis of covariance (20). The paired t test at =0.05 was used to examine the significance of the observed differences. The data are reported as mean $\pm$ SD.

4.3. Results and Discussion

Figure 4-1 depicts chromatograms of brain samples before and 2 h after ip injections of TBZ and DHT. The average time courses of TBZ and DHT in the brain and serum following ip administrations of TBZ and DHT are shown in Figure 4-2. Similar to serum, the maximum brain



Fi[illegible] 1. Chromatograms of brain samples before (A), and 2 h after ip injection of 3 mg/kg of tetrabenazine (B) or dihydrotetrabenazine (C). Peaks: 1, tetrabenazine; 2, dihydrotetrabenazine; 3, internal standard.

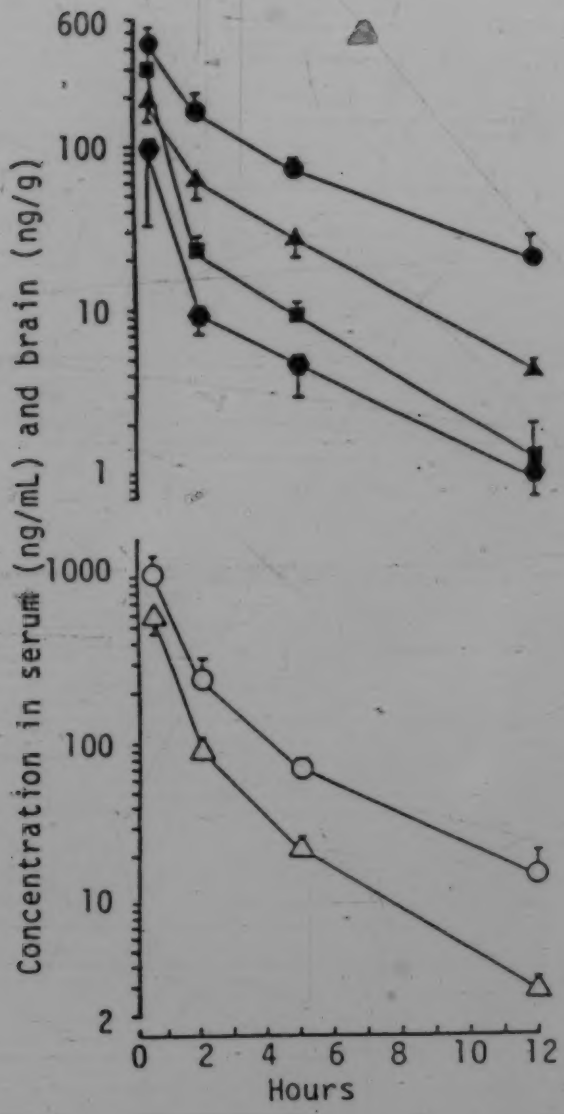


Fig. 4-2. Mean time courses of tetrabenazine and dihydrotetrabenazine after 3 mg/kg single ip doses of the drug (top) and the metabolite (bottom). Key: circles, dihydrotetrabenazine in brain; triangles, dihydrotetrabenzine in serum; squares, tetrabenazine in brain; hexagons, tetrabenazine in serum. Vertical bars represent SD (n = 4).

the brain concentrations is parallel to that in serum, which suggests that the brain is a part of the central compartment. Following administration of both compounds, strong correlations were found between serum and brain concentrations of the drug and the metabolite ($r>0.958$). No tetrabenazine was found in serum or brain following administration of DHT.

The site of action of TBZ is in the central nervous system. Consequently, DHT can elicit in vivo activity only if it crosses the blood-brain barrier (BBB) and/or is formed from TBZ within the brain. Presence of DHT in the brain after its administration (Figures 4-1 and 4-2) indicates that DHT, despite its greater polarity than TBZ, is capable of crossing BBB. The possibility of the formation of the metabolite from tetrabenazine inside the brain, however, remains to be proven.

The observed brain:serum concentration ratios of DHT were 3.16 ± 1.35 and 3.26 ± 1.72 after the administration of TBZ and DHT, respectively. The difference between these two ratios was insignificant, indicating that presence of TBZ in the brain had no effect on the brain:serum concentration ratios of DHT. A brain:serum concentration ratio of 2.15 ± 0.85 was observed for TBZ. The greater brain:serum concentration ratio for DHT as compared to TBZ may be attributed to a difference in binding of these compounds to the plasma or brain proteins. In humans, the plasma protein binding of the metabolite has been shown to be less extensive than that of the parent drug (13). This, however, does not exclude the possibility of a more extensive binding of DHT to the brain proteins.

In plasma of patients taking oral doses of tetrabenazine, Mehvar et al. (16) observed at least 3 unknown compounds, presumably representing other metabolites of the drug. The very high fluorescence intensities of these peaks were attributed to their high plasma concentrations. In the brain, however, only the fluorescence intensities of the peaks representing TBZ and DHT were significant (Figure 4-1).

The effects of TBZ and DHT on the concentrations of monoamines are depicted in the Figure 4-3. Both TBZ and DHT were capable of decreasing brain levels of the amines. Their effects, however, were more intensive on DA than NE and 5-HT; the latter was least affected by TBZ and DHT. Consistent with their maximum serum and brain concentrations, the maximum pharmacologic effects of both TBZ and DHT occurred within 0.5 h and, except for DA, the brain levels of NE and 5-HT returned to their normal values within 5 h. The brain levels of the DA 12 h after injection of either TBZ or DHT, although insignificant, tended to be greater than the baseline values (Figure 4-3). This increase might be attributed to the compensatory mechanisms or to the blockade of presynaptic DA receptors (9).

The percent reductions in the monoamine levels at any time following metabolite injection were greater than the corresponding value after injection of the same dose of the parent drug (Figure 4-3). The difference, although in some instances insignificant, may be attributed to the greater concentrations of DHT achieved after injection of DHT, compared to those observed as a result of parent drug injection (Figure 4-2). These observations suggest that the catecholamine- and 5-HT-depleting effects observed following

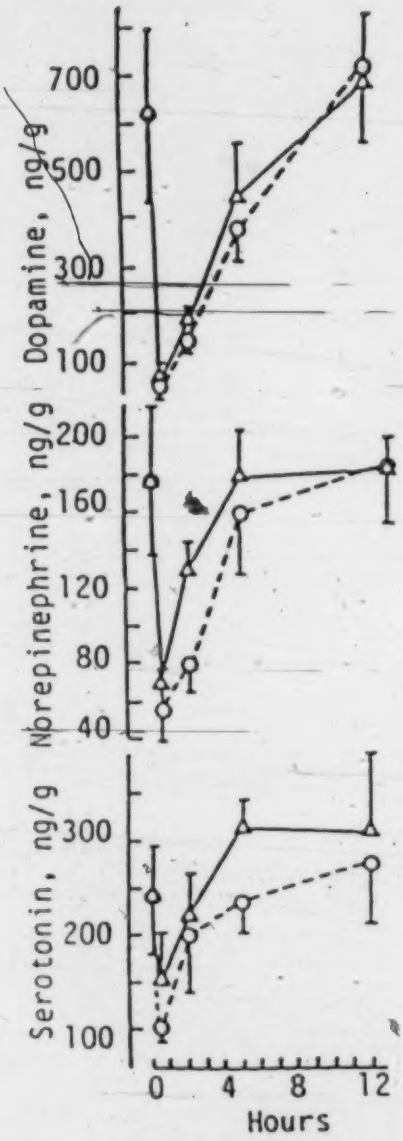


Fig. 4-3. Mean time courses of monoamines in brain after ip injections of 3 mg/kg of tetrabenazine (△) and dihydrotetrabenzine (○). Vertical bars represent SD (n = 4).

administration of tetrabenazine may be due to formation of its dihydrometabolite, and that contribution of the parent drug to these effects is, at most, minimal. Similar to our observations, rapid reductions in the brain levels of DA, NE, and 5-HT were previously reported in rats receiving sc doses of 0.5-10 mg/kg of TBZ; the maximum effect (70-75% reduction in DA with 2 mg/kg dose) was observed 15 min following drug administration (21). After iv injections of 30 mg/kg of DHT to rabbits, Pletcher et al. observed a decrease in the brain levels of NE and 5-HT (7). To our knowledge, in vivo depletion of DA by DHT, however, has not yet been addressed in the literature.

The plots of monoamine levels in the brain versus log serum concentrations of DHT after ip injections of TBZ or DHT showed characteristics of sigmoidal log concentration-response relationships (19) (Figure 4-4). The same relationships were found when the levels of the amines were plotted versus log brain concentrations of DHT. Examination of Figure 4-4 reveals that the sigmoidal curves representing monoamine levels and serum DHT concentrations after TBZ are superimposable with those obtained after DHT. This indicates that the monoamine depletion observed after TBZ, mainly, if not entirely, is due to the formation of DHT.

The regression parameters obtained from the plots of E versus time are listed in Table 4-1. The y-intercept of such a plot is an indication of the maximal effect attainable by that particular dose if administered intravenously (19). In the present study, both the drug and the metabolite were injected via ip route. Consequently, the calculated y-intercepts (Table 4-1) are not precise estimates of the

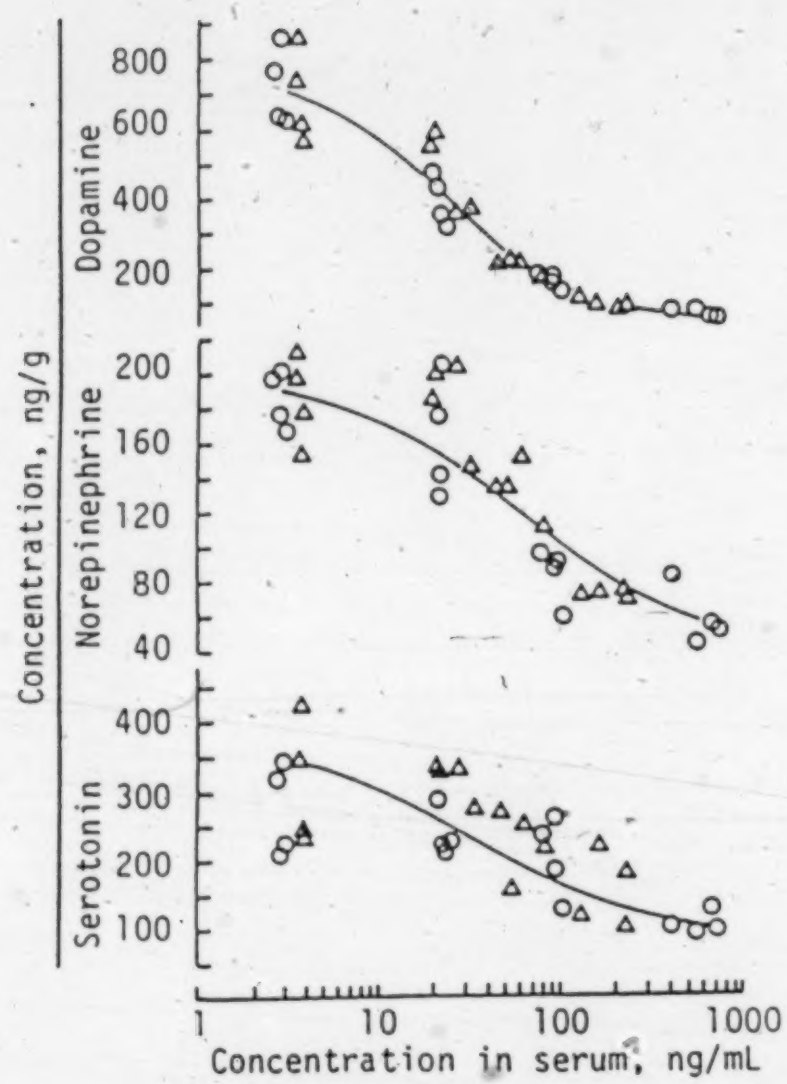


Fig. 4-4. Response versus log serum concentrations of dihydrotetrabenazine after ip injections of tetrabenazine (△) or dihydrotetrabenazine (○).

Table 4-1.Regression parameters[a]

	r		slope		y-intercept(%)	
	TBZ	DHT	TBZ	DHT	TBZ	DHT
DA	0.999	0.998	-13.4	-11.7	96.6	98.7
NE	0.970	0.992	-13.3	-13.5	61.4	78.1
5-HT	0.996	0.905	-14.7	-11.2	43.2	54.3

[a] Obtained from the plots of %effect versus time following single ip injections of 3 mg/kg of tetrabenazine (TBZ) or dihydrotetrabenazine (DHT).

maximum attainable effects after 3-mg/kg doses of TBZ or DHT. This parameter, however, may be utilized for comparison of the potency of TBZ and DHT. As in all cases, the y-intercepts were greater following administration of DHT, one may suggest a potency greater than the unchanged drug for the metabolite. The slopes of the lines, however, were not statistically different (Table 4-1). This might indicate that, in both cases, the same moiety was responsible for the amine-depleting activity observed after ip injections of TBZ or DHT.

In plotting C/E versus serum and brain concentrations of DHT for DA, only data points obtained at 0.5 and 2 h after injection of TBZ and DHT were used (Figure 4-5). Some of the 5-h and all of the 12-h samples had DA concentrations equal to the baseline values. This made the estimation of the exact time at which the levels reached the baseline values impossible. Incorporation of 5- and 12-h data points, therefore, could have distorted the pattern of the dependency of C/E upon C. The E_{max} and K_d values calculated from these plots are presented in Table 4-2. Closeness of the values following administration of TBZ and DHT again suggests that the active moiety in both cases is DHT, not TBZ, and that the presence of TBZ does not affect the affinity or maximum effect produced by DHT.

It has been shown in vitro that tetrabenazine is capable of inhibiting active ATP-dependent transport mechanism of catecholamines (22,23) in the chromaffin granules, the catecholamine storage organelles. The drug also appears to deplete DA and NE from crude preparations of hypothalamic and striatal synaptosomes (24). The ability of dihydrotetrabenazine to bind to the membrane of purified

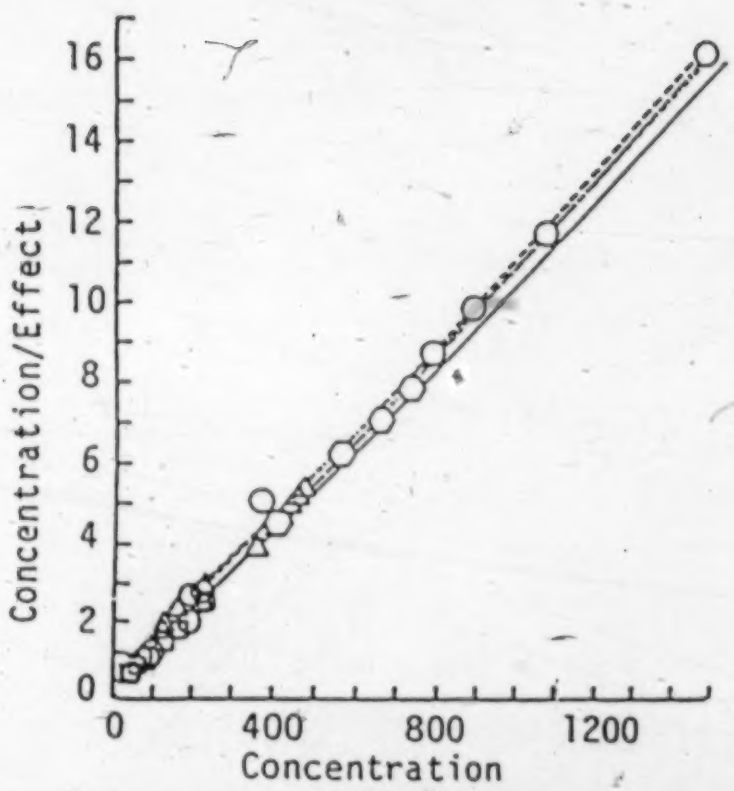


Fig. 4-5. Serum or brain dihydrotetrabenazine concentration /effect (%reduction in brain dopamine level) versus serum (—□—, ···⬡··· ;ng/mL) and brain (–○–,··△··;ng/g) concentration of dihydrotetrabenazine following ip administration of 3 mg/kg tetrabenazine (··△··,—□—) or dihydrotetrabenazine (–○–,···⬡···).

Table 4-2. Maximum effect (E_{max}) and equilibrium dissociation constant (K_d) values after single doses of tetrabenazine (TBZ) or dihydrotetrabenazine (DHT)[a]

Drug	K_d		E_{max} (%)	
	Plasma, ng/mL	Brain, ng/g	Plasma,	Brain,
TBZ	23.8	71.0	100	103
DHT	22.7	50.8	95.7	96.2

[a] Obtained from plots of brain or plasma concentrations of DHT:effect (percent reduction in the brain levels of dopamine) versus concentration after ip doses of 3 mg/kg of TBZ or DHT.

bovine chromaffin granule, and to inhibit NE uptake has also been demonstrated (15,22,23). Scherman and Henry (14) investigated the effect of pH on the in vitro activity of TBZ and DHT, and suggested that at pH 7.5, tetrabenazine is twice as active as DHT in binding to chromaffin granules. They attributed their finding to the difference between pKa of TBZ and DHT, as only the neutral form was assumed to be able to bind to the membrane (14). These in vitro results suggest that the monoamine-depleting activity observed following administration of tetrabenazine is, at least, mainly due to the unchanged drug; a postulate contrary to our in vivo findings. This discrepancy may be due to a more complex in vivo distribution process compared to an in vitro situation. Pletcher (25) also noticed such a discrepancy between in vivo and in vitro results and suggested that in situ, the degree of transportation of the various drugs across the neuronal membrane is different; this results in different intraneuronal drug concentrations. Such differences in the intraneuronal concentrations of TBZ and DHT, therefore, might explain the discrepancy between our results and those obtained in vitro.

In addition to depletion of brain monoamines, TBZ is reported to block both pre- and post-synaptic DA receptors (8-10). This conclusion has been based both on in vivo and in vitro observations following the administration of TBZ. In vitro reports indicate that in rats, TBZ displaces [^{3}H]spiperone from binding to the striatal DA receptors with reported apparent inhibitory constants (Ki) of 5.2 uM (8) and 2.1 uM (9). Reches et al. (9) were able to observe the DA-receptor blockade following ip administration of 5 mg/kg of TBZ. Considering the relatively high Ki values mentioned above, an ip dose

of TBZ>5 mg/kg is expected to be required in vivo to block the DA receptors. Consequently, formation of a more potent metabolite following in vivo administration of TBZ was postulated as one possibility (9). Nevertheless, as in this study, we compared only the monoamine-depleting activities of TBZ and DHT, the blockade of DA receptor by DHT remains to be proven.

In rats, the reduction in spontaneous exploratory behaviour as a result of tetrabenazine therapy has been correlated with the DA and NE depletion (26). In patients with TD, the clinical effects of tetrabenazine seem to be short lasting (27), as is its depleting effect in the rat. Our study suggests that in the rat, the monoamine-depleting activity observed after administration of TBZ is due to the formation of the active metabolite, dihydrotetrabenazine. If these results are extrapolated to man, then, a relatively high plasma concentration of the dihydro metabolite rather than a very low concentration of the parent drug, observed following po administration of tetrabenazine (12,13), may be held responsible for clinical effects.

Our present investigation in the rat shows that the monoamine-depleting activity observed following administration of either the drug or the preformed metabolite is well related to the brain or serum concentrations of the metabolite. Further investigations on patients treated with tetrabenazine for different abnormal movement disorders may also reveal existence of a therapeutic plasma concentration range for the metabolite, dihydrotetrabenazine. The appropriateness of substituting dihydrotetrabenazine for tetrabenazine, as suggested earlier (13),

however, may not be justified before conducting further pharmacokinetic studies on the metabolite, given as such to humans.

4.4. References

1. D. L. McLellan, R. J. Chalmers, and R. H. Johnson: A Double-Blind Trial of Tetrabenazine, Thiopropazate, and Placebo in Patients with Chorea. Lancet 1, 104-107 (1974).

2. D. Kingston: Tetrabenazine for Involuntary Movement Disorders. Med. J. Aust. 1, 628-630 (1979).

3. S. W. Asher, and M. J. Aminoff: Tetrabenazine and Movement Disorders. Neurology 31, 1051-1054 (1981).

4. J. Jankovic: Treatment of Hyperkinetic Movement Disorders With Tetrabenazine: A Double-Blind Crossover Study. Ann. Neurol. 11, 41-47 (1982).

5. S. Fahn: Long-Term Treatment of Tardive Dyskinesia With Presynaptically Acting Dopamine-Depleting Agents. Adv. Neurol. 37, 267-276 (1983).

6. J. F. Marti-Masso and J. A. Obeso: Coprolalia Associated With Hemiballismus: Response to Tetrabenazine. Clin. Neuropharmacol. 8, 189-190 (1985).

7. A. Pletscher, A. Brossi, and K. F. Gey: Benzoquinolizine Derivatives: A New Class of Monoamine Decreasing Drugs With Psychotropic Action. Int. Rev. Neurobiol. 4, 275-306 (1962).

8. I. S. Login, M. J. Cronin, and R. M. MacLeod: Tetrabenazine Has Properties of a Dopamine Receptor Antagonist. Ann. Neurol. 12, 257-262 (1982).

9. A. Reches, R. E. Burke, C. M. Kuhn, M. N. Hassan, V. R. Jackson, and S. Fahn: Tetrabenazine, an Amine Depleting Drug, Also Blocks Dopamine Receptors in Rat Brain. J. Pharmacol. Exp. Ther. 225, 515-521 (1983).

10. I. S. Login, M. J. Cronin, C. T. Harcus, and R. M. MacLeod: Neuroendocrine Evidence That Tetrabenazine Is a Dopamine Antagonist. Proc. Soc. Exp. Biol. Med. 172, 225-231 (1983).

11. D. E. Schwartz, H. Bruderer, J. Rieder and A. Brossi: Metabolic studies of Tetrabenazine, a Psychotropic Drug in Animals and Man. Biochem. Pharmacol. 15, 645-655 (1966).

12. R. Mehvar, F. Jamali, M. W. B. Watson, and D. Skelton: Pharmacokinetics of Tetrabenazine and Its Major Metabolite in Man and Rat. Bioavailability and Dose Dependency Studies. Drug Metab. Dispos. 15, 250-255 (1987).

13. M. S. Roberts, S. McLean, K. S. Millingen, and H. M. Galloway: The Pharmacokinetics of Tetrabenazine and Its Hydroxymetabolite in Patients Treated for Involuntary Movement Disorders. Eur. J. Clin. Pharmacol. 29, 703-708 (1986).

14. D. Scherman, and J. P. Henry: Acido Basic Properties of the Catecholamine Uptake Inhibitors Tetrabenazine and Dihydrotetrabenazine. Biochimie **64**, 915-921 (1982).

15. D. Scherman P. Jaudon, and J. P. Henry: Characterization of Monoamine Carrier of Chromaffin Granule Membrane by Binding of [2-^{3}H]dihydrotetrabenazine. Proc. Nat. Acad. Sci. **80**, 584-588 (1983).

16. R. Mehvar, F. Jamali, M. W. B. Watson, and D. Skelton: Direct High-Performance Liquid Chromatography of Tetrabenazine and Its Metabolite in Plasma of Humans and Rats. J. Pharm. Sci. **75**, 1006-1009 (1986).

17. R. P. S. Kwok, A. V. Juorio: Concentration of Striatal Tyramine and Dopamine Metabolism in Diabetic Rats and Effects of Insulin Administration. Neuroendocrinology **43**, 590-596 (1986).

18. B. J. Gudzinowicz, B. T. Younkin, and M. J. Gudzinowicz: "Drug Dynamics for Analytical, Clinical, and Biological Chemists" Marcel Dekker, New York (1984).

19. M. Gibaldi, and D. Perrier: "Pharmacokinetics" Marcel Dekker, New York (1982).

20. J. H. Zar " Biostatistical Analysis" Prentice-Hall, N.J. (1974).

21. D. J. Pettibone, J. A. Totaro, and A. B. Pflueger: Tetrabenazine-Induced Depletion of Brain Monoamines: Characterization and Interaction With Selected Antidepressants. Eur. J. Pharmacol. **102**, 425-430 (1984).

22. D. Scherman, and J. P. Henry: The Catecholamine Carrier of Bovine Chromaffin Granules: Form of the Bound Amine. Mol. Pharmacol. **23**, 431-436 (1983).

23. D. Scherman, and J. P. Henry: Reserpine Binding to Bovine Chromaffin Granule Membranes: Characterization and Comparison with Dihydrotetrabenazine Binding. Mol. Pharmacol. **25**, 113-122 (1984).

24. G. S. Takimoto, J. D. Stittsworth, Jr., B. R. Bianchi, and J. K. Stephens: Differential Sensitivity of Hypothalamic Norepinephrine and Striatal Dopamine to Catecholamine-Depleting Agents. J. Pharmacol. Exp. Ther. **226**, 432-439 (1983).

25. A. Pletscher: Effect of Neuroleptics and Other Drugs on Monoamine Uptake by Membranes of Adrenal Chromaffin Granules. Br. J. Pharmacol. **59**, 419-424 (1977).

26. C. Onn-Leng, R. A. Webster: Effects of Tetrabenazine and .-methyl-m-tyrosine on Exploratory Activity and Brain Catecholamines in Rats. Br. J. Pharmacol. **41**, 691-699 (1971).

27. M. W. B. Watson, personal communication.

5. GENERAL DISCUSSION AND CONCLUSIONS

Prior to the present investigation, the following conclusions could be deduced from the literature on TBZ:

1. Tetrabenazine is a promising drug in the control of abnormal movement disorders, especially TD and Huntington's chorea.
2. The clinical effects of the drug appears rapidly after the drug administration, and abnormal movements reappear soon after drug withdrawal.
3. The dose determination of TBZ was empirical, as virtually no information was available on the pharmacokinetics of the drug.

The above information led us to postulate that clinical effects of the drug might be correlated to the concentrations of the drug and/or its metabolite(s) in the body. To examine this postulate, development of a reproducible, specific and sensitive analytic method was necessary. This proved to be a difficult challenge, as plasma concentrations of the drug were in the low ng/mL range.

Having overcome the difficulties associated with the determination of the drug and its major metabolite, DHT, in biological samples, the pharmacokinetics of the drug and DHT were investigated in the patients and rats. The results of the latter study indicated that TBZ is almost totally metabolized in the liver in both species. It was also concluded that because of a high extraction ratio, a majority of the administered oral dose is metabolized during the first pass through the liver. The concentrations of the dihydro metabolite, in both species, were severalfold higher than those of the parent drug.

Based on the relatively high concentrations of the dihydro metabolite, the question then was whether TBZ or DHT was responsible for the clinical effects of TBZ administration. The last part of the present report revealed that in the rat the pharmacologic activities of TBZ administration were indeed related to the formation of the active metabolite, DHT. An excellent correlation between plasma and brain concentrations of the dihydro metabolite suggests that in the patients, there might exist a therapeutic plasma range for the metabolite.

The results of our study indicate that tetrabenazine might be a potential candidate for therapeutic drug monitoring. Considering the relatively large intra- and inter-individual variations in the metabolism of highly-extracted drugs such as TBZ, one might expect a more consistent result with DHT rather than TBZ. This suggestion, however, needs further evaluation.